PREVALENCE, RISK FACTORS AND GENETIC RELATEDNESS AMONG THERMOPHILIC CAMPYLOBACTERS FROM HUMANS AND CHICKENS USING RAPD-PCR IN MOROGORO, TANZANIA

BY

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jejuni and 5% of *C. coli* were 100% similar. Conclusively, zoonotic thermophilic campylobacter infections existed between humans and chickens in Morogoro municipality. Besides chickens, the role played by other domestic and wild animals in transmitting thermophilic campylobacter infections to humans particularly children, should be closely examined.

DECLARATION

I, Idrissa Shomari Chuma, do declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has not been nor concurrently being submitted for a higher degree award in any other university.

05/05/2009

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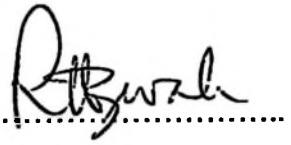
Date



07/05/2009

Prof. R. R: Kazwala
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Date

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DEDICATION

This work is dedicated to my beloved wife, Mwanaisha Hamza Kionga and our firstborn daughter, Fatma I. S. Chuma for their patience, encouragement and hospitality. They are wonderful!

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ABBREVIATIONS AND SYMBOLS

AFLP	-	Amplified fragment length polymorphism
AIDS	-	Acquired immunodeficiency syndrome
CDT	-	Cytolethal distending toxin
c.f.u.	-	Colony-forming unit
Corp.	-	Corporation
DNA	-	Deoxyribonucleic acid
Dnase	-	Deoxyribonuclease
EDTA	-	Ethylene diaminetetraacetic acid
ET(s)	-	Electrophoretic type(s)
FVM	-	Faculty of Veterinary Medicine
GBS	-	Guillain-Barré Syndrome
HCl	-	Hydrochloric acid
HIV	-	Human immunodeficiency virus
IV	-	Intravenous
KCl	-	Potassium chloride
MOMP	-	Major outer membrane protein
MVM	-	Master of Veterinary Medicine
NART	-	Nalidixic acid resistant test
NIMR	-	National Institute for Medical Research (Tanzania)
NVH	-	Norges veterinærhøgskole (Norwegian School of Veterinary Science)
OPD	-	Out patient department

PCR	-	Polymerase chain reaction
RAPD	-	Random amplified polymorphic DNA
RFLP	-	Restriction fragment length polymorphism
RNA	-	Ribonucleic acid
rRNA	-	Ribosomal RNA
SUA	-	Sokoine University of Agriculture
Spp	-	Species
Subsp	-	Subspecies
TBE	-	Tris-base Boric acid and EDTA mixture
Tris	-	Trizma
UK	-	United Kingdom
UMB	-	Universitetet for miljø-og biovitenskap (Norwegian University of Life Sciences)
USA	-	United States of America
VBNC	-	Viable but non-culturable
v/v	-	Volume by volume
w/v	-	Weight by volume

CHAPTER ONE

1. INTRODUCTION

1.1. Background

The term *Campylobacter* was derived from Greek words *campylos* and *bacter*, which mean 'curved' and 'rods', respectively (Kazwala, 1988; Crushell *et al.*, 2004). The term 'campylobacteria' refers to fastidious, mainly spiral or curved-rod-shaped bacteria that belong to phylogenetically related genera *Campylobacter*, *Arcobacter*, and *Helicobacter* (On, 1996). Campylobacter species are gram negative and flagellated bacteria classified under the genus *Campylobacter*, family Campylobacteriaceae. These pathogens cause zoonotic infectious diseases generally referred to as campylobacteriosis. Since 1970s, *Campylobacter jejuni* subspecies *jejuni*, *C. coli* and *C. lari* have been documented to cause gastroenteritis, which is a major public health concern worldwide (Nachamkin and Blaser, 2000). These three campylobacter species have been referred to as 'thermophilic' because of their optimum growth at 42°C. Campylobacter species are ubiquitous and may be isolated from gastrointestinal tract of mammals, birds and environments.

Natural reservoirs of campylobacter include chicken and other poultry, wild birds, pigs, dogs, cats, sheep and cows among others (Stern, 1992; Workman *et al.*, 2005). Human infection occurs through consumption of contaminated food and water or by direct contact with infected animals. Clinical spectrum of campylobacter enteritis ranges from watery, non-bloody, non-inflammatory diarrhea to a severe inflammatory diarrhea with abdominal pain and fever (Coker *et al.*, 2002).

Antibiotics such as erythromycin and ciprofloxacin are drugs of choice for treatment of campylobacter enteritis in both humans and animals (Engberg *et al.*, 2001). Prevention and control measures for campylobacter infection include: reducing contamination of food, thorough cooking of food, pasteurization of milk, using potable drinking water, avoiding consumption of raw foods, and public education on environmental and personal hygiene.

Urban and peri-urban agriculture (UPA) has been expanding in Tanzania. While chicken, cattle and pigs are the major livestock species kept for food purposes; dogs and cats are kept for watching, controlling pests, hunting purposes and as pets. Increasing urbanization with animals living in closer proximity with humans poses risks of contracting zoonotic diseases including campylobacteriosis. Spread of zoonotic diseases may also be enhanced by other factors including: poor hygiene and sanitation; malnutrition; poor health status; poor feeding habits; poor immunity and HIV and AIDS (Altekruse *et al.*, 1999; Rao *et al.*, 2001; Coker *et al.*, 2002; Ali *et al.*, 2003; Rani *et al*, 2004; Kusiluka *et al.*, 2005; Mdegela *et al.*, 2006). For example, Altekruse *et al.* (1999) reported that patients with HIV and AIDS were 39 times more prone to campylobacteriosis than normal healthy individuals in United States. Higher prevalence of HIV and AIDS in developing countries exacerbates the situation (Coker *et al.*, 2002). The number of campylobacter cases in Sub-Sahara Africa is predicted to double by the year 2020 (Coker *et al.*, 2002).

Considering the importance of campylobacter infections worldwide, several typing techniques and their combinations have been used to compare genotypes among campylobacter strains. The isolates involved were recovered from various reservoirs and patients with campylobacter-associated diseases. The techniques used include: flagellin (Fla) typing, multilocus enzyme electrophoresis (MLEE), ribotyping, multilocus sequence typing (MLST), pulsed field gel electrophoresis (PFGE), macrorestriction profiling, amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP).

Using several afore-mentioned typing techniques in different parts of the world, some domestic and wild animal species have been reported to act as sources of human infection. Several epidemiological studies aimed at isolating thermophilic campylobacter species and establish their prevalence in Tanzania have been conducted in humans, poultry and water (Jiwa *et al.*, 1997; Nonga 2005; Mdegela *et al.*, 2006). However, it is still unclear which host species among domestic or wild animals act as a major source of the prevailing thermophilic campylobacter species both in human and animal populations. Furthermore, exploration of genetic similarity or diversity among thermophilic campylobacter isolates from humans and animals has not been attempted in Tanzania. Such exploration could have given clue on source(s) of human infection among domestic animals. Therefore, it is worthy exploring genetic relatedness among thermophilic campylobacter isolates from human and chicken populations in Morogoro municipality so as to know whether zoonotic infections existed and if one of these hosts acted as a reservoir of infections

to the other. The current study will hopefully positively contribute to the existing scientific knowledge and bridge the gap in the genetic epidemiology of thermophilic campylobacter species in Tanzania.

Random amplified polymorphic DNA (RAPD) PCR technique was successfully used as an epidemiological tool to identify sources of campylobacter infection due to its ability to identify specific DNA regions associated with a given phenotype of different microorganisms (Mazurier *et al.*, 1992; Williams *et al.*, 1993; Mondon *et al.*, 1995). Some researchers used RAPD technique to discriminate between invasive and noninvasive clinical isolates and O19-positive from O19-negative *C. jejuni* strains (Missawa *et al.*, 1998; Carvalho *et al.*, 2001). In the current study, the RAPD PCR technique was been chosen and used because it is simple, fast and cost-effective PCR assay that has good ability to identify and discriminate campylobacter isolates from disparate hosts (Missawa *et al.*, 1998; Carvalho *et al.*, 2001; Stepniak *et al.*, 2002; Workman *et al.*, 2005). In addition, BioNumerics computer software was used for computational analysis in determining genetic relatedness among campylobacter isolates. The use of computational analysis increases consistency of this typing technique and comparison of data (Hernandez *et al.*, 1996).

1.2. Objectives

1.2.1. Main objective

The overall objective of this study was to establish whether thermophilic campylobacter species are shared between human and chicken populations (existence of zoonotic infections) in Morogoro municipality, Tanzania.

1.2.2. Specific objectives

Specific objectives of this study were: -

1.2.2.1. To establish prevalence of thermophilic campylobacter species in children under five years and chickens in Morogoro municipality

1.2.2.2. To determine risk factors for thermophilic campylobacter infections in children less than five years and chickens

1.2.2.3. To determine genetic relatedness among thermophilic campylobacter isolates from humans and chickens.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. History of campylobacter species

Different opinions exist among scientists with regard to history of campylobacter species. A German physician, Escherisch observed campylobacter species when he examined stool of infants with diarrhea in 1880 (Trachoo, 2003). *Vibrio fetus* was claimed to be responsible for spontaneous abortions in cattle and sheep. This microorganism differed from Vibrio species in that it could not grow well under atmospheric oxygen tension and did not ferment sugars (Trachoo, 2003). Hansson (2007) believes that McFadyean and Stockman were the first to isolate and identify campylobacter species in 1913, which were causing infertility and abortion in bovine and ovine. For the first time, campylobacter species were described as an important cause of diarrhea after "Vibrio" had been isolated from inflamed intestines of cattle and calves; these microorganisms were termed *Vibrio jejuni* (Jones *et al.*, 1931; cited by Hansson, 2007). Later on, the microorganisms were further renamed *Campylobacters* was whereby '*campylos*' means 'curved' and '*bacter*' stands for 'rods' (Sebald and Veron, 1963; cited by Kazwala, 1988). The renaming aimed at differentiating these bacteria from *Vibrio* species (Catteau, 1995). Kist reviewed the work of Escherisch in 1985 and confirmed presence of spiral shaped organisms that were similar to campylobacter species (Kazwala, 1988).

The first isolation of campylobacter spp. from humans was done in 1938 in Illinois, USA, where there were two outbreaks of diarrhea thought to be milk-borne (Levy, 1946; cited by Hansson, 2007). According to Hansson, (2007), in 1957 and 1962, King worked on bacteria isolated from late 1950s and managed to isolate thermophilic campylobacter species. King confirmed that those bacteria were the cause of gastroenteritis though their isolation from diarrhoeic patients was still difficult (Hansson, 2007). In 1972, isolation of *Campylobacter* spp. from blood and stool of a young woman with acute febrile haemorrhagic enteritis was successfully done in Belgium (Dekeyser *et al.*, 1972). However, there was little attention until when Skirrow developed a selective medium for direct isolation of campylobacter species. Skirrow isolated campylobacter species from a febrile and diarrhoeic baby in 1977 (Hansson, 2007). The medium contained antimicrobials to suppress other faecal microorganisms while favouring growth of campylobacter species. *Campylobacter jejuni* was filtered and then cultured on blood-thioglycolate agar medium mixed with bacitracin, polymixin B sulphate, novobiocin and actidione to take care of bacterial and fungal contaminants (Trachoo, 2003).

Since then, there has been a rise of campylobacter infection over those of Salmonellosis and Shigellosis (Altekruse *et al.*, 1999). Therefore, thermophilic campylobacter species have become important leading agents of human gastroenteritis in various parts of the world (Blaser and Reller, 1981; Tauxe *et al.*, 1985; Skirrow, 1987).

2.2 Scientific classification

Classification of members of the genus Campylobacter based on 16S rRNA gene was described by Hansson (2007). Classification of campylobacter species and subspecies was done by various scientists and compiled by Euzéby (1997). At present, the data show that the genus *Campylobacter* contains 26 species and 11 subspecies as shown in Table 1 (Euzéby, 1997). Briefly, Hansson (2007) classified campylobacter as follows:

Domain: *Bacteria*

Phylum: *Proteobacteria*

Class: *Epsilonproteobacteria*

Order: *Campylobacteriales*

Family: *Campylobacteriaceae*

Genus: *Campylobacter*

2.2. Campylobacteriosis

Campylobacteriosis is a collective description for infectious diseases caused by members of bacterial genus Campylobacter. The most important form is campylobacter enteritis, which is mostly caused by a group of thermophilic campylobacter species - *C. jejuni* and *C. coli* (Nachamkin and Blaser, 2000). However, *C. lari* is rarely found (Kazwala *et al.*, 1990). These pathogenic species are found in many warm-blooded mammals and birds as normal flora. Carriage of campylobacter species by apparently healthy animals threatens human health (Nachamkin and Blaser, 2000) as the animals act as sources of infection to humans.

Campylobacteriosis is an occupational infection with people in close contact with animals and birds being at high risk of infection (Ellis *et al.*, 1995). Infants in developing countries are challenged by *C. jejuni* (Coker *et al.*, 2002).

Table 1. Campylobacter species and subspecies

No.	Species	Subspecies
1	*Campylobacter butzleri*	-
2	*Campylobacter canadensis*	-
3	*Campylobacter cinaedi*	-
4	*Campylobacter coli*	-
5	*Campylobacter concisus*	-
6	*Campylobacter cryaerophilus*	-
7	*Campylobacter curvus*	-
8	*Campylobacter fennelliae*	-
9	*Campylobacter fetus*	*Campylobacter fetus* subsp. *fetus*
		Campylobacter fetus subsp. *venerealis*
10	*Campylobacter gracilis*	-
11	*Campylobacter helveticus*	-
12	*Campylobacter hominis*	-
13	*Campylobacter hyoilei*	-
14	*Campylobacter hyointestinalis*	*Campylobacter hyointestinalis* subsp. *hyointestinalis*
		Campylobacter hyointestinalis subsp. *lawsonii*
15	*Campylobacter insulaenigrae*	-
16	*Campylobacter jejuni*	*Campylobacter jejuni* subsp. *doylei*
		Campylobacter jejuni subsp. *jejuni*
17	*Campylobacter lanienae*	-
18	*Campylobacter lari*	-
19	*Campylobacter mucosalis*	-
20	*Campylobacter mustelae*	-
21	*Campylobacter nitrofigilis*	-
22	*Campylobacter pylori*	*Campylobacter pylori* subsp. *mustelae*
		Campylobacter pylori subsp. *pylori*
23	*Campylobacter rectus*	-
24	*Campylobacter showae*	-
25	*Campylobacter sputorum*	*Campylobacter sputorum* subsp. *bubulus*
		Campylobacter sputorum subsp. *mucosalis*
		Campylobacter sputorum subsp. *sputorum*
26	*Campylobacter upsaliensis*	-

Source: Euzéby (1997)

2.3. Aetiology

A group of thermotolerant campylobacter species comprising of *C. jejuni*, *C. coli* and *C. lari*, are the major cause of campylobacteriosis (Nachamkin and Blaser, 2000). *Campylobacter jejuni* is the most frequently isolated species from cases of humans with campylobacterial diarrhoea accounting up to 90% of all infectious cases of human campylobateriosis (Koenraad, 1995; Blaser, 1997). *Campylobacter lari* is rarely found (Engvall *et al.*, 1986; Kazwala *et al.*, 1990). This might partly be a reflection of the isolation media used as there is no one media that can recover all campylobacter species (Saleha *et al.*, 1998). Species other than *C. jejuni* and *C. coli*, such as *C. upsaliensis*, *C. jejuni* subsp. *doylei*, *C. fetus* subsp. *fetus*, *C. concisus* and other campylobacter-related organisms such as *Arcobacter butzleri*, *Arcobacter cryaerophilus*, *Helicobacter fennelliae* and *Helicobacter cinaedi* are also of public health importance (Lindblom, *et al.*, 1995a; On, 1996). However, lack of diagnostic capacity hinders availability of epidemiological data on these other species in developing countries (On, 1996; Vandenberg *et al.*, 2001).

Campylobacter jejuni also has been implicated in a number of conditions affecting animals such as diarrhoea in dogs, cats, zoo animals, calves and horses (Sandberg, 2002). *Campylobacter jejuni* was, further more, reported to cause mastitis in cows, avian infectious hepatitis and abortions in sheep and cows (Carter and Chengappa, 1991; Skirrow and Butzler, 2000; Misawa *et al.*, 2002).

2.4. Eco-environmental survival and distribution

Campylobacter species demonstrate considerable ecological diversity (On, 1996) that is incompletely understood (Skelly and Weinstein, 2003). These bacteria are of clinical and economic importance due to infections they cause (On, 1996). Wild and domestic animals, especially poultry potentially harbour the pathogens as part of normal gut flora (Al-Mashat and Taylor, 1980; Prescott and Bruin-Mosch, 1981; Hald and Madsen, 1997). Campylobacter species replicate almost exclusively within the intestinal tract of warm-blooded animal hosts (Mangia *et al.*, 1993). Their dispersal from the animal host is through excretion in faeces or contamination of carcass by intestinal contents during slaughter (Skelly and Weinstein, 2003). However, their movement and persistence through the environments is less clearly known.

Many animal species are reported to harbour and probably involved in the spread of thermophilic campylobacter species. Apart from avian species, other animals involved in the spread of these pathogens include: dogs, cats, goats, pigs, rats, shellfish such as clams, seagulls, crows, blue magpies, sparrow and grey starlings (Rosef, 1981; Kapperud and Rosef, 1983; Kasrazadeh and Genigeorgis, 1987; Ito *et al.*, 1988; Franco, 1989; Yogasundram *et al.*, 1989; Butzler and Oosterom, 1991; Skirrow, 1991; Raji *et al.*, 2000; Mdegela *et al.*, 2006). Insects, in particular houseflies, have been shown to play a role in dissemination of campylobacter infection in chickens (Rosef and Kapperud 1983; Shane *et al.*, 1985). Campylobacter

species have also been isolated from the genital, untreated drinking water and anaerobic sludge (Laanbroek *et al.*, 1977; Blaser and Reller, 1981; Penner, 1988).

2.5. Transmission and risk factors

Examination of animals, aquatic environments and food-related surfaces indicate that there is an abundance of animal reservoirs and variety of survival trajectories through the environment (Skelly and Weinstein, 2003). Ingestion of contaminated foods of animal origin, drinking untreated water, and direct contact with infected animals especially pets are the main sources of human infection (Blaser and Reller, 1981). Sources of campylobacter infection includes: inadequately cleaned and disinfected poultry house; polluted water particularly if not chlorinated; reuse of old litter; pests; feed; insects like houseflies and darkling beetles; lesser mealworm; rodents and free-flying birds accessing to poultry houses (Shane *et al.*, 1985; Genigeorgis *et al.*, 1986; Engvall *et al.*, 1986; Kazwala *et al.*, 1990; van de Giessen *et al.*, 1993; Saleha *et al.*, 1998). The transmission cycle showing various suspected sources of infection and virulence determinants has been depicted in Fig. 1.

Feathers, skin and intestines of colonized birds leads to contamination of equipment, working surfaces and process water in poultry processing plant (Oosterom *et al.*, 1983; Genigeorgis *et al.*, 1986). Campylobacter dose sufficient to infect humans varies from 500 to 100 000 organisms but may be as low as 4 cfu/g of meat (Oosterom *et al.*, 1983; Butzler and Oosterom, 1991). However, higher level of *C. jejuni* of up to 10^3 - 10^5 cfu per processed carcass has been reported (Oosterom *et al.*,

1983). This amount is relatively higher than commonly reported infective dose (Saleha *et al.*, 1998).

Evidences have been documented in different parts of the world on domestic animal species and their products acting as the sources of human campylobacter infections. For example, Siemer *et al.* (2004) showed that dogs were a source of human infection in Denmark. Similarly, Workman *et al.* (2005) concluded that dogs and chicken meat are the most likely sources of human campylobacteriosis in Barbados.

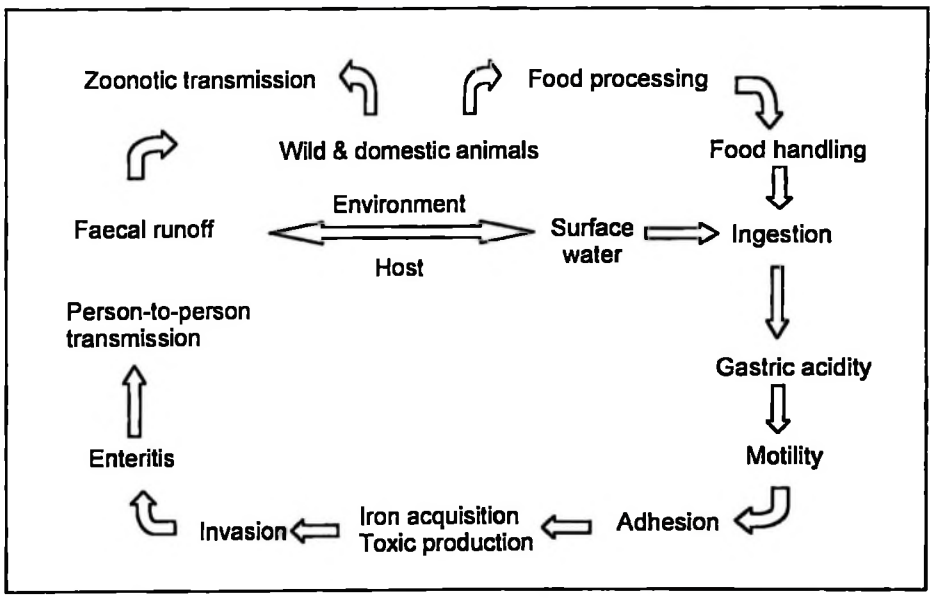

Figure 1: A model of the transmission cycle of *C. jejuni* depicting suspected environmental, food and animal sources and putative virulence determinants for infection of susceptible hosts (Altekruse and Tollefson, 2003).

Farm staff may also spread campylobacter species by hands, boots/footware and clothing (Lindblom *et al.*, 1986; Kazwala *et al.*, 1990). Travel to a developing country was reported as a risk factor for acquiring campylobacter-associated diarrhoea (Coker *et al.*, 2002). For example, Mattila *et al.* (1992) observed that campylobacter enteritis was more prevalent in winter months among the tourists who visited Morocco. On the other side, vertical transmission of *C. jejuni* via the egg is considered unlikely, partly because of low rate of isolation of campylobacter from naturally or experimentally infected eggs (van de Giessen *et al.*, 1993; Jacobs-Reitsma, 1995). After associating the isolation of *C. jejuni* serotype in broiler chickens with a particular hatchery that supplied the chicks, Pearson *et al.* (1993) suggested re-examination of vertical transmission.

Campylobacter species die relatively rapidly under adverse environmental conditions. However, their survival may be enhanced by formation of dormant cells that are viable but unable to multiply in conventional culture media (Rollins and Colwell, 1986). This phenomenon of viable but non-culturable (VBNC) cells has been a matter for debate among microbiologists as its role in transmitting campylobacter infections among poultry is yet to be clarified (Saleha *et al.*, 1998). At present, it is difficult to distinguish between non-viable, viable but non-culturable and sublethal injured campylobacter species (Saleha *et al.*, 1998).

2.6. Pathogenesis and virulence tools

Motility, chemotaxis and flagella are known to be important factors in the virulence as they are required for attachment and colonization of intestinal epithelium (Ketley, 1997). In addition, adhesion, invasion, toxin production, and subversion of host cell processes are themes common to the virulence tools of many enteric prokaryotic pathogens (Crushell *et al.*, 2004). Once colonization has occurred, campylobacter bacteria may disturb the normal absorptive capacity of the intestine. This is achieved by damaging epithelial cell functions either directly by cell invasion and/or production of toxin(s). Disturbance of the absorptive capacity may also be caused indirectly following initiation of an inflammatory response (Wooldridge & Ketley, 1997). Several virulence determinants have been involved in induction of diarrhea. These factors include: adhesion and invasion molecules, outer membrane proteins, lipopolysaccharides, stress proteins, flagella and motility, M cells, iron acquiring mechanisms, and cytotonic and cytotoxic factors (Smith, 1996). However, relative role and importance of these factors in development of diarrhoea is not quite clear (Christensen *et al.*, 2001). The ability of campylobacter species to invade host cells in vitro is well established and cytotoxin production is consistently reported (Ketley, 1997).

2.6.1. Flagella

The flagella of *C. jejuni* are composed of proteins, *FlaA* and *FlaB*, encoded by two genes sharing a high degree of sequence homology (Wassenaar and Blaser, 1999). Disruptions in *FlaA*, in particular, reduce motility and markedly reduce the ability of

the organism to colonize the gastrointestinal tract (Wassenaar and Blaser, 1999). However, flagella are also important for invasion of (and to a lesser extent adherence to) host cells, as aflagellate organisms show markedly reduced internalization into host cells *in vitro* (Wassenaar and Blaser, 1999).

2.6.2. Invasion

Campylobacter jejuni has been shown within human colonic epithelial cells taken both from infected humans and macaque monkeys (Wooldridge and Ketley, 1997). In addition, the invasiveness of this organism has been studied in a variety of cell lines, in particular human intestine-derived Caco-2 and INT 407 cells (Wooldridge and Ketley, 1997; Kopecko *et al.*, 2001). Although some isolates of *C. jejuni*, such as the well characterized 81–176 strain, are highly invasive in these experimental models, many isolates show low levels of host cell entry *in vitro* (Kopecko *et al.*, 2001). Campylobacter species may not efficiently enter the host cell via the apical membrane, and recent evidence supports the contention that *C. jejuni* preferentially enters polarized epithelial cells via the basolateral membrane (Monteville and Konkel, 2002). In support of this model, there exists evidence both for paracellular passage (Monteville and Konkel, 2002) and M-cell transcytosis of *C. jejuni* (Wooldridge and Ketley, 1997; Kopecko *et al.*, 2001).

Many invasive pathogens subvert host cytoskeletal structures as part of the pathogenic process (Crushell *et al.*, 2004). Highly invasive *C. jejuni* 81–176 demonstrates microtubule-dependent invasion and rely on microtubule motors for

uptake and intracellular motility (Bourke, 2002; Hu and Kopecko, 1999). Nonetheless, most strains of *C. jejuni* demonstrate microfilament-dependent or microfilament/microtubule-dependent invasiveness (Biswas *et al.*, 2000). Little is known of the invasiveness of other enteric campylobacter species. However, a recent report implicates both of these cytoskeletal structures during cellular uptake of *C. upsaliensis* (Mooney *et al.*, 2003).

Campylobacter jejuni attach to the apical surface of host intestinal cells after access to, and motility within the mucus layer (Fig. 2, step A). Current evidence suggests that the attachment process is mediated by adhesin-receptor interactions. Putative adhesins include major outer membrane protein (MOMP), lipooligosacharride, capsular polysaccharide, CadF, JlpA, and PEB1. *Campylobacter jejuni* is among a handful of bacteria that subvert host microtubule structures to gain entry to and move within host cells. Bacterial secreted proteins may be involved in recruiting host cell structures including microtubules, microtubule motors (*e.g.* dynein), and microfilaments. The proteins may also play part in inducing host cell protein tyrosine phosphorylation or activation of trimeric G proteins for bacterial entry into the cell and possible subsequent intracytoplasmic motility (Monteville and Konkel, 2002).

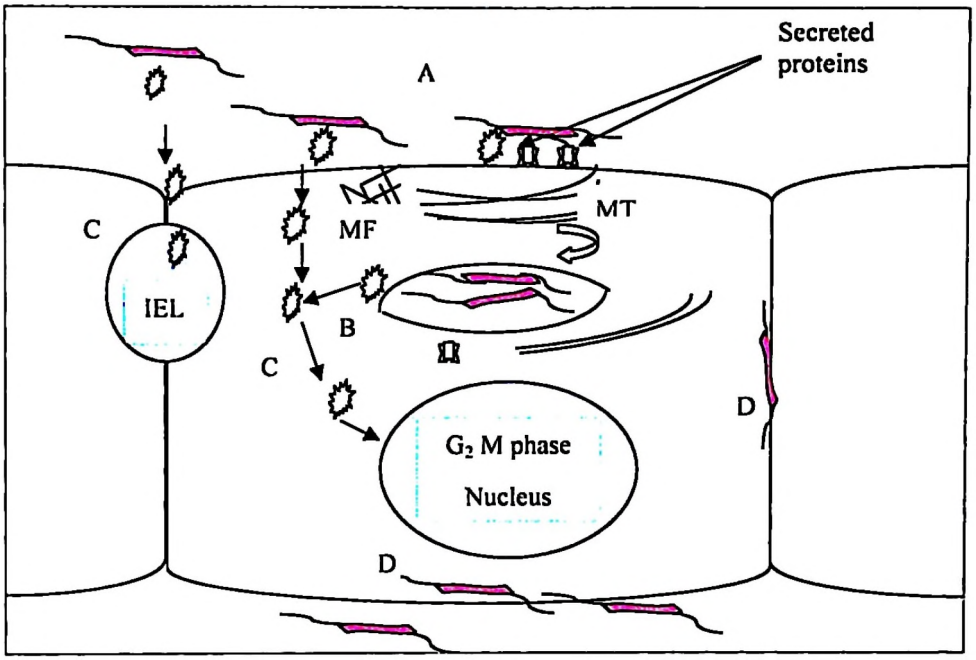

Figure 2: A speculative model of *C. jejuni* interaction with host intestinal epithelial cells: MT, microtubules; MF, microfilaments; IEL, intraepithelial lymphocytes, cytolethal distending toxin (B subunit), secreted effector proteins including CiaB (Monteville and Konkel, 2002).

Campylobacter jejuni survive and replicate within intracytoplasmic vacuoles, at least to some extent (Fig. 2, step B). Access of these bacteria to adjacent cells is unknown. *Campylobacter jejuni* strains make at least one cytotoxin, cytolethal distending toxin (CDT) which has DNase activity mediated by CdtB (Pickett, 2000). The cytotoxin act directly on enterocytes to induce host cell cycle arrest, distension, and proinflammatory cytokine induction (Fig. 2, step C). Alternatively, CDT may cause cell cycle arrest by acting on immune cells such as intraepithelial lymphocytes. Whether CDT accesses cells after release from bacteria before cell contact, at the

time of attachment, or after internalization is unknown. Paracellular migration of bacteria (Fig. 2, step D) possibly through disrupted tight junctions, or M-cell transcytosis with access of bacteria to the basolateral membrane of the enterocytes also is supported by some data (Monteville and Konkel, 2002).

2.6.3. Adhesion

A variety of putative *C. jejuni* adhesins have been identified. These adhesins include PEB1 (a homolog of Gram-negative ABC transport systems) (Pei and Blaser, 1993), CadF (a fibronectin-binding protein) (Konkel *et al.*, 1997), major outer membrane protein (Moser *et al.*, 1997), and lipooligosaccharide (Fry *et al.*, 2000). Recently, a novel surface-exposed lipoprotein specific to *C. jejuni* has been implicated in host cell adherence (Jin *et al.*, 2001). Contribution of these potential virulence factors to human disease is still unknown. Several studies have been hampered due to lack of a suitable and widely accessible animal model (Crushell *et al.*, 2004). According to Crushell *et al.* (2004), adherence of *C. jejuni* to host cells is a multistep process, involving both specific and nonspecific adhesin-receptor interactions. Intriguingly, *C. jejuni* genome sequence has revealed genes responsible for biosynthesis of capsular polysaccharides. This heretofore unrecognized structure is common among *C. jejuni* strains, and appears to form the basis of the Penner serotyping system for this species (Karlyshev and Wren, 2001). Recent publications also suggest a role for this polysaccharide structure in virulence (Karlyshev and Wren, 2001; Bacon *et al.*, 2001). Presence of hypervariable genes within the locus with the potential for phase

variation (Karlyshev and Wren. 2001) is notable in this respect (Crushell *et al.*, 2004).

2.6.4. Toxin production

Historically, a variety of toxic activities have been attributed to *C. jejuni*. This subject has been reviewed by Pickett (2000). However, cytolethal distending toxin (CDT) is the only verified campylobacter toxin identified to date (Monteville and Konekel, 2002). Work on CDT from campylobacter species and other organisms recently has led to a rapid advancement in understanding cellular effects of these proteins *in vitro* (Hickey *et al.*,1999; Pickett, 2000; Elwell and Dreyfus, 2000; Hickey *et al.*, 2000; Purdy *et al.*, 2000; Karlyshev and Wren, 2001; Bacon *et al.*, 2001). Now it is clear that CdtB is the active moiety of Cdt ABC complex and *cdtB* gene product has DNasc activity (Lara-Tejero and Galan, 2001). The CdtA and CdtC interact with CdtB to form a tripartite CDT holotoxin necessary for the delivery of the enzymatically active subunit, CdtB (Lara-Tejero and Galan, 2001).

Affected epithelial cells undergo cytodistension and cell cycle arrest in G_2/M phase (Elwell and Dreyfus, 2000; Lara-Tejero and Galan, 2001). Cell cycle arrest also occurs in T-lymphocytes exposed to CDT-mediating sonicates from *C. upsaliensis* (Mooney *et al.*, 2001) but the role played by CDT in causing infection *in vivo* is not clear (Crushell *et al.*, 2004). However, it has been proposed that CDT may contribute more to immune-modulation and invasiveness (Purdy *et al.*, 2000) than being involved in direct induction of diarrhea.

2.6.5. Secreted proteins and chemokine induction

Subversion of host cell processes by targeting bacterial products to host cytoplasm during the infection process is an increasingly recognized prokaryotic virulence mechanism. Using confocal microscopy, Konkel *et al.* (1999) identified a *C. jejuni* protein, CiaB that appears to enter host cells during the invasion process. Isogenic ciaB-negative mutants were shown to be deficient in secretion of a number of bacterial proteins. The genome of *C. jejuni* 11168 encodes a flagellar export system. However, there is no evidence for the presence of a typical type III secretory apparatus (also termed as 'molecular syringe') implicated in delivering bacterial proteins to the host cell in other infections. Recently, homologs of a type IV secretory apparatus have been identified on a large plasmid (pVir), which is a 37-kb in *C. jejuni* 81–176 (Bacon *et al.*, 2000). Type IV systems e.g cag pathogenicity island also are used by bacterial pathogens including *H. pylori* to inject substrates into the cytosol of target cells. The plasmid pVir of *C. jejuni* harbors 54 predicted open reading frames (Bacon *et al.*, 2002). Mutations in some of the plasmid-encoded genes reduced invasion compared with the parental strain *in vitro*. However, according to Bacon *et al.* (2000), transfer of the plasmid to the sequenced strain NCTC 11168, could not enhance its relatively low invasiveness. Furthermore, with observation that only 10% of 58 fresh clinical *C. jejuni* isolates harbored the plasmid-encoded VirB11 gene, pVir is not responsible for all *C. jejuni*-induced pathogenic effects.

Enterocolitis due to *C. jejuni* involves activation of the local immune response. Interleukin IL-8 is a potent chemokine that can be induced by *C. jejuni,* is involved

in neutrophil attraction, among other things (Hickey *et al.*, 1999). Current evidence implicates both direct interaction of *C. jejuni* with host cells and CDT elaboration in the induction of IL-8, higher levels of which requires uptake of bacteria into the host cells (Hickey *et al.*, 1999; Hickey *et al.*, 2000).

Understanding pathogenesis of *C. jejuni* has apparently been rapidly expanding. Recently, successful and efficient transposon mutagenesis has breached one of the early barriers to the explication of *C. jejuni* molecular pathogenesis (Colegio *et al.*, 2001; Hendrixson *et al.*, 2001). These developments herald a renaissance period for campylobacter research that can be expected to continue for some time (Crushell *et al.*, 2004). Together with these developments, genomic sequence will provide the cornerstone for powerful novel techniques such as microarray and protein expression methodologies.

2.7. Clinical manifestation and complications

2.7.1. Humans

In most cases, campylobacteriosis afflicts more children both under and above five years, the elderly and immunocompromied individuals than healthy normal adults. The incubation period is between 2 to 5 days. The infection is normally self-limiting and lasts one to four days or up to 10 days at the most (Saleha *et al.*, 1998). The sickness begins with a fever associated with malaise and headaches followed with nausea and abdominal cramping resembling the symptoms of acute appendicitis

(Catteau, 1995; Altekruse *et al.*, 1999; Saleha *et al.*, 1998). Patients in developing countries appear to have less severe symptoms than those in developed countries (Trachoo, 2003). For example, in developed countries the disease is characterized by bloody stool, fever and abdominal pain that is often severe than that observed in Shigella and Salmonella infections (Coker *et al.*, 2002). In developing countries, features reported are watery stool, abdominal pain, vomiting, dehydration, and presence of faecal leucocytes; patients are also often under-weight and malnourished (Coker and Dosunmu-Ognubi, 1985; Bhadra *et al.*, 1989; Rao *et al.*, 2001). Bloody diarrhoea was reported in fifty percent of patients in developed world as opposed to 15% in developing countries (Taylor *et al.*, 1993).

Occasionally, *C. jejuni* infection leads to bacteremia, septic arthritis and other complications. Rarer still are meningitis, recurrent colitis, acute cholecystitis and Guillain-Barré Syndrome (GBS) (Skirrow, 1992). Interestingly, extra-intestinal infections such as pericarditis and myocarditis have recently been increasingly reported as complications associated with campylobacter infection (Uzoigwe, 2005). Although this has been rarely found, due care is needed to avoid thrombolysis or angioplasty, which may be brought about as a result of inappropriate diagnosis (Uzoigwe, 2005). Common complications of campylobacter infection are persistent infection and infection with antimicrobial resistant strains of campylobacter in AIDS patients (Perlman *et al.*, 1988). GBS, which is reported to link to and succeed *C. jejuni* infection (Nachamkin, 1998; Coker *et al.*, 2002), is an autoimmune disorder of the peripheral nervous system. GBS is characterized by acute flacid, rapid

progressive symmetric paralysis (Coker *et al.*, 2002; Trachoo, 2003). Sporadic cases of GBS have been reported from various parts of the world such as Curacao, China, India and South Africa (Lastovica *et al.*, 1997; van Koningsveld *et al.*, 2001). Detailed studies on the role of GBS in acute flaccid paralysis are needed in developing countries, especially in polio-endemic areas (Coker *et al.*, 2002). Campylobacteriosis is also associated with Reiter Syndrome, a reactive arthropathy thought to be caused by autoimmune responses. Reiter Syndrome is stimulated by campylobacter infection and pathogenesis of the autoimmune responses is not completely understood (Saleha *et al.*, 1998). According to Saleha *et al.* (1998), sterile post-infection process occurs 7 to 10 days after onset of diarrhoea in approximately 1% of patients. Multiple joints especially the knee can be affected and pain and incapacitation can last for months or become chronic.

2.7.2. Chickens

Chickens are healthy carriers of thermophilic campylobacter species in their gastrointestinal tracks though sometimes they can be pathogenically affected. There is no appreciable development of clinical signs in chickens because, in most cases, they are healthy carriers of these bacteria. Colonization refers to benign, non-pathological commensal relationship that exists between host and an organism or parasite (Saleha, 2004). For enteric organisms like *C. jejuni* to establish and maintain colonization, a complex interaction of unknown mechanisms between the host and the organism comes into play (Stern, 1994). *Campylobacter jejuni* is introduced and spreads rapidly to virtually all birds in the flock (Lindblom *et al.*, 1986; Stern, 1992).

The spread is facilitated by contamination of feed and water (Genigeorgis *et al.*, 1986). Main sites of colonization of *C. jejuni* are the caeca and the organisms are concentrated in the mucus layer in the crypts of the villi (Beery *et al.*, 1988). At these predilection sites, campylobacter species are usually free-living and have ability to utilize mucin as an energy source (Beery *et al.*, 1988). Levels of colonization are relatively highly detectable in faeces and concentrations of 5.6×10^4 to 1.2×10^7 cfu/g have been reported (Grant *et al.*, 1980).

Some broiler flocks appear to stay free from campylobacter species until they are sent for slaughter, which is usually at the age of six weeks or more (Genigeorgis *et al.*, 1986; Lindblom *et al.*, 1986). Some strains of this species are invasive and/or toxigenic and may cause distention of intestines with contents tend to become foamy, liver abnormalities and diarrhoea (Clark and Bueschkens, 1988). Such strains can also be isolated from spleen, gall bladder and blood of infected chickens (Beery *et al.*, 1988). These facts supports observation by van de Giessen *et al.* (1993) who suggested that *C. jejuni* should not be regarded as part of the normal intestinal microflora of poultry.

2.8. Diagnosis and strain discrimination

Like any other enteropathogens, clinical picture of campylobacter enteritis varies from asymptomatic secretion or mild symptoms to severe disease in humans. Bacterial culture is considered as a gold standard diagnostic method as it is used to isolate various pathogens from their hosts - humans, chickens and other animals.

However, in absence of culture facilities or in cases of clinical urgency, rapid presumptive diagnosis can be made by direct faecal examination using a microscope after performing Gram's staining technique. Campylobacter species are Gram negative, small (0.2-0.8 μm wide and 0.5-5 μm long), spiral or curved rods. Dark field or phase contrast microscopy makes it possible to appreciate a characteristic darting or cork-screw like motility. Polymerase chain reaction (PCR), which is a DNA-based technique, has been described (Altekruse *et al.*, 1999) and is nowadays used for both diagnostic and research purposes. Like other pathogens, *C. jejuni* infection raises serum antibodies but detectable levels are usually noticed after symptoms have resolved. This is a drawback to the use of serology as a diagnostic tool in humans.

Genotypic methods have nowadays been increasingly used for diagnostic and research purposes in various parts of the world. These methods have ability to discriminate thermophilic campylobacter species and strains. Using various typing techniques, genetic diversity within campylobacter species has been documented. For examples, Meinersmann *et al.* (2002) and Acik and Cetinkaya (2006) typed *C. jejuni* and *C. coli* isolates from disparate hosts and concluded that high degree of heterogeneity existed among these pathogens. Some typing techniques used for discriminating thermophilic campylobacter species and/strains in different studies are highlighted hereunder:

2.8.1 Flagellin (Fla) typing

Campylobacter jejuni has flagellin gene locus that contains two flagellin genes (*flaA* and *flaB*). These genes are arranged in tandem and are separated by approximately 170 nucleotides (Wassenaar and Newell, 2000). Presence of both highly conserved and variable regions makes restriction fragment length polymorphism (RFLP) analysis of a PCR product from this locus possible (Meinersmann *et al.*, 1997). In most cases, only one *fla* gene is amplified but weak bands are sometimes visible. As Primers designed for *C. jejuni* may be used to genotype related pathogens like some strains of *C. coli*, *C. lari*, and *C. helveticus*, *which* have partially conserved regions at this locus (Owen *et al.*, 1993). Many isolates can be easily compared, especially if international standardization of at least primers and restriction enzymes used (Wassenaar and Newell, 2000). *Fla* typing profiles can be readily stored in electronic databases (Nachamkin *et al.*, 1996).

2.8.2 Pulsed Field Gel Electrophoresis (PFGE)

Very large DNA fragments (20 to 200 kb) obtained from restriction can be separated basing on size by using special electrophoretic conditions (Wassenaar and Newell, 2000). Genotypic or macrorestriction profiles are obtained as a result of action of restriction enzymes on various restriction sites. Bacterial suspensions are immobilized in agarose before the cells are lysed to protect chromosomal DNA from shearing. All subsequent enzymatic steps are carried out by means of passive diffusion into the agarose blocks so that any plasmids are detected by PFGE together with the chromosomal DNA. Purified and digested DNA are directly loaded into

agarose gel blocks and electrophoresed in electrical field that is changed in a pulsed manner. Although more than one enzyme increases discriminatory power, comparisons of the PFGE profiles obtained in different laboratories have proved to be difficult (Wassenaar and Newell, 2000).

2.8.3 Ribotyping

Campylobacter chromosome has multiple copies of the rRNA gene loci coding for 5S, 16S and 23S rRNA at different positions. Strong conservation of regions in the rRNA genes and highly variable (noncoding) flanking regions renders it suitable for subtyping. Agarose gel electrophoresis of digested genomic DNA followed by Southern blot hybridization with a probe specific for rRNA genes is the commonly used ribotyping technique (Wassenaar and Newell, 2000). Though typeability is high, the discriminatory power of this method is limited as most *campylobacter* spp. contain only three ribosomal gene copies. Differences in the restriction enzymes and probes used generally hamper direct comparisons of the ribotype profiles obtained in different laboratories. Relatively low discriminatory power, high cost, low throughput and the elaborate nature of this technique make it a relatively unsuitable for routine genotyping (Wassenaar and Newell, 2000).

2.8.4 Multilocus Enzyme Electrophoresis (MLEE)

MLEE has contributed most to understanding of the global epidemiology and population structure of infectious agents as high levels of discrimination are achieved by analyzing many loci (Maiden *et al.*, 1998). This method successfully identifies clusters of closely related strains (clones or clonal complexes) that are particularly

liable to cause disease. However, results obtained in different laboratories are difficult to compare (Maiden *et al.*, 1998).

2.8.5 Multilocus sequence typing (MLST)

MLST is a simple technique that requires only the ability to amplify DNA fragments by PCR and sequence them using an automated sequencer or manually. Adapting concepts and methods of MLEE, MLST can identify alleles directly from the nucleotide sequences of internal fragments of the housekeeping genes (Maiden *et al.*, 1998). Examination of multi loci is important as clones diversify with age, as a consequence of mutations or recombinations (Maiden *et al.*, 1998). More variation and many alleles per locus can be detected, sequence data can be compared readily between laboratories, and sequences of gene fragments from a number of different housekeeping loci is fully portable and the data be easily accessed electronically on the internet.

2.8.6 Random amplified polymorphic DNA (RAPD)

RAPD analysis amplifies random DNA segments with a primer of arbitrary nucleotide sequences under low-stringency and generates genotype-specific banding patterns (Welsh and McClelland, 1990). PCR products are produced when primer sites are located within the amplification distance (less than 5 kb) with correct opposite orientation (Welsh and McClelland, 1990). The lengths of these products, efficiency of annealing and thus amplification vary with the sites primed, hence, production of both strong and weak amplicons (Wassenaar and Newell, 2000).

RAPD technique has been reported to be a useful tool for scanning and picking differences from the entire genome (Missawa *et al.*, 2000). Computational analysis of band patterns may improve consistency of this technique and comparisons of data; however, there are still arbitrary decisions on whether weaker bands should be ignored or not (Hernandez *et al.*, 1996). Despite development of RAPD methods for *Campylobacter* spp., up to 14% of the strains may be untypeable due to DNase activity (Hernandez *et al.*, 1996). Major disadvantage of RAPD analysis is poor reproducibility as minor differences in band patterns can lead to subjective interpretation (Wassenaar and Newell, 2000). The differences may occur even when purified DNA is used as the template. This considerably complicates interpretation of the results. Though the actual reasons for variations are not known, some implicated possible causes are inconsistencies in thermal cyclers, template purity and procedures (Ellsworth *et al.*, 1993; MacPherson *et al.*, 1993; Penner *et al.*, 1993). In addition, computer-aided representations may simulate higher reproducibility than can be obtained in reality (Hernandez *et al.*, 1996).

2.9. Public health concerns and future management perspectives

Campylobacter infection is an occupational disease among farm workers; this may be due to close contact of the workers and farm animals (Ellis *et al.*, 1995). Jones and Robinson (1981) reported that 27-68% of personnel in poultry and red meat abattoirs had complement fixing antibodies compared with 3% of the rural field labourers. Free-range chickens maintained in close association with humans are recognized as

potential source of *C. jejuni* infection (Marquis *et al.*, 1990; Rao *et al.*, 2001). A study in Peru showed that children in families that kept infected chickens were 12 times more likely to contract enteritis than those in households without chickens (Marquis *et al.*, 1990). While contamination of animal products threatens consumers' health, preventing animals from being colonized by these microorganisms is cumbersome and perplexing challenge to scientists all over the world.

Campylobacter species afflict children at different levels worldwide. In developing countries, incidences between 40 000 to 60 000 out of every 100 000 children below five years have been estimated as compared to 300 for every 100 0000 children in developed countries (Tauxe, 1992; Taylor, 1992; Oberhelman and Taylor, 2000; Rao, *et al.*, 2001). The cost for health care and loss of productivity was estimated at British Pounds, £ 273 (about US $ 480) in the developed world (Skirrow and Blaser, 1992). This loss is equivalent to Tanzanian Shs. 576 000.

Enteritis due to *C. jejuni* is, in most cases, mild and self-limiting. Erythromycin and ciprofloxacin are drugs of choice for treatment of severe or prolonged cases but gentamicin, norfloxacin or nalidixic acid can also be used (Engberg *et al.*, 2001). However, since *C. jejuni* was recognized as a pathogen, reports of its antimicrobial resistance to drugs including have increased (Naeem and Macaulay, 1983; Wickin *et al.*, 2001). Extensive use of antimicrobial agents in human and food animals is a most probable cause of the increase in antimicroabial resistance (Piddock, 1995; Aarestrup and Wagener, 1999). Reports that fluoroquinolones such as ciprofloxacin,

temafloxacin and norfloxacin may be of limited use for campylobacter infections in humans have raised concerns (Engberg *et al.*, 2001).

Some scientists opined that campylobacteriosis is not severe in developing than in developed countries (Taylor, 1992; Oberhelman and Taylor, 2000). However, given the high incidence of HIV and AIDS cases in developing countries, campylobacter-associated diarrhea, bacteremia and other clinical manifestations are also higher in HIV and AIDS patients than in HIV-negative patients (Coker *et al.*, 2002). Similarly, campylobacter infections cause substantial mortality and morbidity in HIV and AIDS patients than in HIV-negative patients (Coker *et al.*, 2002). Furthermore, HIV and AIDS contribute substantially to deaths among children less than five years in epidemic settings (Adetunji, 2000). Thus, infants in developing countries are at risk of impaired immunity to *C. jejuni* enteritis (Coker *et al.*, 2002). Further more, HIV and AIDS increase the number of campylobacteriosis in adult population in these countries (Coker *et al.*, 2002). These observations in developing countries (Coker *et al.*, 2002) and the burden in these countries may increase by 2020 due to HIV and AIDS epidemic (Coker *et al.*, 2002).

CHAPTER THREE

3. MATERIALS AND METHODS

This study was conducted between December 2006 and January 2007 to find out whether zoonotic thermophilic campylobacter infections existed in human and chicken populations in Morogoro municipality. The aim of the current study was to determine the prevalence, identify risk factors for infection and describe genetic relatedness among thermophilic campylobacter isolates from humans and chickens.

3.1. Study area

This study was conducted in Morogoro municipality (an urban area) in Tanzania. According to data obtained from Tanzania Meteorological Agency (Morogoro office), Morogoro municipality is extended between longitude 35°15' to 38°30' East and latitude 5°15' to 10° 0' South on slopes of Uluguru Mountains at an altitude of 526.0 m above sea level. The municipality has a bimodal rainfall pattern with 188.5-409.4mm and temperature ranges from 15.0 to 33.1 °C. Short rains fall from November to January and the long rains around March to May. Administratively, the municipality has one division that is organized into 19 wards and 274 streets. The population and housing census (United Republic of Tanzania, 2002) showed that the municipality has a population of 228 863 people (113 639 Males and 115 224 Females). The number of children less than five years (from zero to four years) was 27 629, of which 13 964 were males and 13 665 females. Data from Morogoro Municipal Health Office showed that there were 76 health facilities of which 4 were

hospitals, 13 health centres, 38 dispensaries and 21 laboratories. Also, data obtained from Municipal Agriculture, Livestock Development and Cooperatives Office revealed that a total of about 2.25 million chickens were kept by farmers during the time of this study.

3.2. Study design, inclusion criteria and sample size

Cross-sectional study design was used to determine prevalence and risk factors associated with thermophilic campylobacter infections in humans and chickens. In the case of human study, children below five years attending various health care facilities for different medical attentions were targeted. Several health care facilities were proposed but only those whose officers-in-charge accepted were actually involved. Children attending Outpatient Department (OPD) at Mafiga Health Centre, Morogoro Regional and Mazimbu Hospitals were enrolled. Other enrolled were those taken to Madizini, Usangi and Upendo Medical Laboratories for medical examination. Antibiotic therapy, admission and hospitalization were regarded as confounding factors and children involved in any of these were excluded. Also children aged less than 3 months were excluded to avoid confounding effects of maternal immunity. On the other side, chicken farms were purposively selected and only farms whose owners accepted were enrolled. Chickens less than three weeks of age were excluded as maternal immunity could have influenced the prevalence. Chickens that were currently undergoing treatment with antibiotics (from the day of sampling up to 7 days back) were also excluded to avoid confounding the actual prevalence.

Sample sizes were calculated using the formula $n=Z^2p\ (1-p)/\ d^2$ (Thrusfield, 1995) where: n is sample size; Z is the multiplier from the normal distribution, p is the expected prevalence and d is the desired absolute precision. The expected prevalence of campylobacter infection (p) used for sample size estimation was p=20% for humans (Lindblom *et al.*, 1995b) and p=70% for chickens (Nonga, 2005). Other values (Z, d, and CI) were kept constant. With Z value of 1.96 at 95% confidence interval (CI) and desired precision (d) of 0.05, the calculated minimum sample sizes (n) were 250 and 330 for humans and chickens, respectively.

3.3. Sample sources

3.3.1. Humans

A list of healthcare facilities in Morogoro municipality was obtained from Morogoro Municipal Office and six health facilities were randomly selected. Faecal samples were collected from children less than five years attending any of the six health care facilities for any reason. The health facilities were: Morogoro Regional and Mazimbu Hospitals, Mafiga Health Centre, and Upendo, Usangi and Madizini Medical Laboratories. The faecal samples/stools were collected from children attending Outpatient Departments (OPD) and their counterparts attending the previously mentioned medical laboratories.

3.3.2. Chickens

Cloacal swabs were collected from chickens (indigenous, broilers, layers and cockerels) of different age in selected farms or flocks. These were commercial birds except the indigenous chickens. A total of 22 chicken flocks/farms located in various areas within Morogoro municipality were involved in this study. Chickens for sale at the Morogoro Central Market were also included. For convenience, flocks with one to 199 chickens were classified as small, 200-299 medium and 300-7 000 large.

3.4. Sampling

3.4.1. Humans

A list 76 health facilities was obtained from Morogoro Municipal Health Office and each facility was assigned a unique number. With the aid of a computer, random numbers were generated and the health facilities selected basing on order of appearance of its corresponding number. For convenient sample collection work and laboratory analysis that were done on daily basis, only six (6) health facilities were selected. Human samples were collected by medical laboratory technicians working with health facilities concerned. While at the health facility, clean 10ml-plastic containers known as securitainers (Conplas, Exports c. c., Ferndale, South Africa) were provided to parents or guardians of study children for stool collection. About 3g or 3 ml of faecal samples/stools were collected and submitted to the technicians. The technicians aseptically picked a loopful of each sample and put into 5ml of campylobacter enrichment broth contained in a sterile 10ml-universal bottles and tightly but securely covered them. Besides biodata, risk factors associated with

human infection such as age (months), sex, keeping chickens, keeping other animals and boiling or treating drinking water were also sought and recorded in sample submission forms. Medical laboratory technicians asked for this information from the parents/guardians.

3.4.2. Chickens

A list of chicken farms/flocks was obtained from the Municipal Agriculture, Livestock Development and Cooperatives Office in Morogoro. Each farm/flock was assigned a unique number generated from computer-aided randomization. Twenty two (22) chicken farms/flocks were randomly selected and each farm or flock was visited for chicken sampling purposes. The chickens were randomly picked and sampled while making a transect walk across the chicken barn as recommended by Noordhuizen *et al.* (2001). Sterile cotton swabs were moistened by being soaked briefly into campylobacter enrichment broth and then inserted gently and carefully into the cloaca/recta via the vents. The swabs were rolled gently in different directions while held against cloacal mucosa and then scooped out together with smears of cloacal contents. Thereafter the swabs were immediately placed in 5ml of fresh campylobacter enrichment broth contained in sterile 10ml-universal bottles and their covers were securely tightened. To avoid sampling the same bird more than once, sampled chickens were temporarily separated until sampling process was over.

Risk factors associated with chicken infection including chicken types, flock size, age and keeping other animals were sought and/recorded in sample collection forms.

Age of chickens was determined basing on farmers records. However, due to difficulties to determine specific age of indigenous local chickens, the age of some chickens was determined by approximation basing on body size. Each chicken was judged accordingly whether it was a chick or an adult. Only the adult indigenous chickens were sampled but the chicks were left out. Flock size and keeping of other animals were also determined basing on farmer's responses, records and direct observation of the flocks.

3.5. Sample handling and transportation

After collection and before transported to the Public Health Laboratory at the Faculty of Veterinary Medicine, Sokoine University of Agriculture in Morogoro for further processing. Human faecal samples/stools were refrigerated at 4°C for few minutes to about three hours prior to transport. Chicken faecal swabs were collected and immediately put in universal bottles containing 5ml of Campylobacter Enrichment Broth (Lab M, International Diagnostics Group, plc, Lancashire, UK). All chicken cloacal swabs and human stool samples were placed on ice blocks in a cool box at 4°C and transported from farms or health care facilities to the laboratory within two hours.

3.6 Isolation of campylobacter species

As part of quality control, a preliminary work involving sampling, isolation and identification of thermophilic campylobacter species was done under supervision of competent and experienced scientists prior to this study. In the current study, the

isolation of thermophilic campylobacter species from children and chickens was done using media prepared as per manufacturers' instructions as elaborated below.

3.6.1 Campylobacter enrichment broth

The Campylobacter enrichment broth (Lab M, International Diagnostics Group, plc, Lancashire, UK) contained the following ingredients: meat peptone 10g/l; lactalbumin hyrolysate 5g/l; yeast extract 5g/l; sodium chloride 5g/l; haemin 0.01g/l; sodium pyruvate 0.5 g/l; a-ketoglutaric acid 1.0 g/l; sodium metabiosulphite 0.5 g/l and sodium carbonate 0.6 g/l. The media was prepared by dissolving 27.6 g in 1litre of distilled water, soaked for 10 minutes and then swirled to mix as per manufacturer's instructions. Autoclaving was done at 121°C for 15 minutes, after which the media was cooled to 47°C and then supplemented with 2 vials of saponin lysed horse blood. The mixture was mixed well and 5ml aseptically dispensed into respective 10ml-universal bottles. The media was left to cool before being incubated at 37°C for 24 hours to check for sterility.

3.6.2 Modified cefoperazone charcoal deoxychocolate agar

Campylobacter blood-free selective agar base (Modified-Preston) (Oxoid Ltd, Basingstoke, Hampshire, England) or Modified cefoperazone charcoal deoxychocolate agar (mCCDA) media was used. This media contained: 25g/l of nutrient broth No. 2; bacteriological charcoal 4g/l; casein hydrolysate 3g/l; sodium desoxychocolate 1.0 g/l; ferrous sulphate 0.25g/l; sodium pyruvate 0.25g/l and agar 12 g/l. The media was prepared by mixing 22.75g with one litre of distilled water, the mixture was then boiled to dissolve and autoclaved at 121°C for 15 minutes as

per manufacturer's instructions. The media was cooled to 40°C and then supplemented with one vials of CCDA selective supplement (Oxoid Ltd, Basingstoke, Hampshire, England). The mixture was mixed well and aseptically dispensed into petri dishes. The media was left to cool and solidify before incubation at 37°C for 24 hours to check for sterility.

3.6.3 Blood agar

Blood agar (Himedia, Himedia Laboratoties Ltd, Mumbai, India) supplemented with 5-10% horse blood (v/v) was used to grow campylobacter species for NART test and sub-culturing of the isolates prior to RAPD PCR genotyping. The media was prepared by dissolving 40g into distilled water, allowing dissolving completely and autoclaving at 121°C for 15 minutes. The media was left at room temperature to cool to 50°C and aseptically mixed with 5% horse blood (v/v). The mixture was gently mixed well and aseptically dispensed into petri dishes and left to cool and solidify before incubation at 37°C for 24 hours to check for sterility.

3.6.4 Brain heart infusion broth

Brain heart infusion broth (Oxoid Ltd, Basingstoke, Hampshire, England) media with 20% glycerol was used for storage of isolates. This was prepared by dissolving 37g in 1litre of distilled water followed by mixing thoroughly. The media was sterilized by autoclaving at 121°C for 15 minutes. After preparation, it was left to cool and sterile glycerol was added to make up 20% of the final volume. The mixture was thoroughly mixed and 1 ml was dispensed aseptically into separate cryogenic vials (Nalgene®, Nalge Nunc Int. Corp, USA).

3.7 Culture and identification of campylobacter species

Campylobacter species are relatively slow-growing, fastidious bacteria that require specialized culture conditions. They grow best under reduced oxygen tension on nutritional basal media supplemented with 5-10% blood. In the laboratory, human stool samples and chicken cloacal swabs were aseptically inoculated in 10ml-universal bottles containing 5ml of Campylobacter Enrichment Broth (Lab M, International Diagnostics Group, plc, Lancashire, UK). The bottles were put in an incubator (Heraeus B5050, Germany) at 37°C for 24 hours.

After enrichment one loopful of the enriched human and chicken samples were plated onto modified cefoperazone charcoal deoxychocolate agar (mCCDA) (Oxoid Ltd, Basingstoke, Hampshire, England) supplemented with CCDA selective supplement (Oxoid Ltd, Basingstoke, Hampshire, England). The plates were put in an anaerobic jar (Coldstream Engeneering Ltd, 18-10, Arista, Sweden) with microacrophilic environments generated by a lighted candle. Thereafter, the plates were put in the incubator (Memmert, Germany) at 43°C for 48 hours.

Bacterial colonies were suspected to be thermophilic campylobacter species based on growth at 43°C and colonial morphology. Campylobacter colonies were: small, mucoid, low convex to flat shaped grey colonies with glossy and sticky or swarming with metallic sheen appearance on fresh media. Considering their margins, the colonies were smooth or rough-edged. When examined under dark-field microscope,

campylobacter species had characteristic rotating rapid corkscrew-like or darting motility. Sometimes when daughter cells remain joined, long spiral forms were seen.

3.7.1 Gram staining

One beautiful suspect colony was picked up using a sterile wire loop and smeared on a glass slide for microscopy. Campylobacter species are Gram negative, slender comma-, spiral- or rod-shaped bacteria. Bacteria with these characteristics were regarded as thermophilic campylobacter species and they were further identified by biochemical tests to species level. Campylobacter species picked up the counter stain weakly and hence, they always stained light-pinkish or red.

3.7.2 Biochemical tests

Campylobacter species neither form spores nor ferment or oxidize carbohydrates. In this study, biochemical tests used to differentiate thermophilic campylobacter isolates from humans and chickens are briefly described below:

3.7.2.1 Oxidase test

Isolated campylobacter species were subjected to oxidase test. Oxidase sticks (Oxoid Ltd, Basingstoke, Hampshire, England) were applied to the suspect bacterial colony and left for a while at room temperature, development of purple colour at the impregnated end of the stick meant a positive reaction. Alternatively, a thick smear of a colony was put on the bloating paper wetted in 1% aqueous solution of *tetramethyl-p-phenylenediamine* and left for few seconds. Immediate development of a deep purple colour around the smear implied a positive oxidase reaction.

3.7.2.2 Catalase test

Campylobacter isolates were also subjected to catalase test. A suspect bacterial colony was subjected to filter paper that had been wet with 3% Hydrogen peroxide solution (Oxoid Ltd, Basingstoke, Hampshire, England) at room temperature. Development of effervescence within few seconds implied a positive reaction.

3.7.2.3 Hippurate hydrolysis

Hippurate hydrolysis differentiates *C. jejuni* from other thermophilic campylobacter species that give negative results. Campylobacter isolates were inoculated into test tubes containing 0.4 ml (400µl) of 1% Hippuric acid (Benzoylaminoacetic acid) sodium salt (Sigma, Aldrich Co. St Louis, USA) solution and incubated (Memmert, Germany) at 37°C for 24 hours. Then 0.2ml (200µl) of 3.5% Ninhydrin (Sigma Grade, Sigma, Aldrich Co. St Louis, USA) solution was added to each test tube and observed for 10 to 20 minutes. Formation of purple colour implied a positive reaction and any other colour meant negative reaction.

3.7.2.4 Nalidixic acid resistance

Nalidixic acid resistance test (NART) differentiates *C. lari* from other thermophilic campylobacter species. *Campylobacter lari* is NART positive while other thermophilic campylobacter species give negative results. Suspected colonies of thermophilic campylobacters were streaked on blood agar (Himedia, Himedia Laboratoties Ltd, Mumbai, India) supplemented with 5% horse blood. Nalidixic acid antibiotic disks (30µg/ml) (Oxoid Ltd, Basingstoke, Hampshire, England) were

applied on the surface of the media and plates were put in an incubator (Memmert, Germany) set at 37°C for 24 hours under microaerophilic condition. Lack of inhibition zone (a clear zone) of any size was regarded as resistance and any inhibition as susceptibility.

3.7.2.5 Nitrate reduction

The isolates were subjected to nitrate reduction test (Lipfilchem, s.r.l, Roseto d.A. (TE) Italy) in order to identify *C. coli* from other campylobacter species. The former gave a positive reaction while the later gave negative reactions.

3.8 Bacterial storage

Confirmed and biotyped thermophilic campylobcater isolates were sub-cultured on mCCDA (Oxoid Ltd, Basingstoke, Hampshire, England) as described in part 3.7. Three loopfuls of 48-hour old campylobacter colonies were transferred into cryogenic vials (Nalgene®, Nalge Nunc Int. Corp, USA) containing 1ml of brain heart infusion broth (Oxoid Ltd, Basingstoke, Hampshire, England) with 20% glycerol. The vials were incubated at 37C for 24 hours, initially stored at -20°C for 24 hours and then at -80°C until required for RAPD-PCR typing.

3.9 RAPD-PCR genotyping

Campylobacter jejuni and *C. coli* isolates from chicken faecal samples were selected for genotyping and compared with isolates obtained from stools of children under five years. The isolates were genotyped by RAPD PCR technique using the primer

OPA 11 (5'-CAA TCG CCG T-3') based on the method described by Hernandez *et al.* (1995).

3.9.1 Extraction of bacterial DNA

Template DNAs were prepared as described by Miwa *et al.* (2003). For each isolate, a suspension of bacterial cells was prepared in sterile distilled water and the suspension was boiled for 10 minutes. Cell debris was pelleted by centrifugation at 14,000 × *g* in a benchtop centrifuge for 1 min and supernatant used as the template DNA.

3.9.2 Mastermix preparation

The RAPD reaction mixture consisted of 50 mM KCl, 10 mM Tris-HCl (pH 8.4 at 25°C), 2.5 mM $MgCl_2$, 0.1% Triton X-100, a 200 µM concentration of each deoxynucleoside triphosphate, 0.3 µM of the primer, 2.5 U of *Taq* DNA polymerase (Invitrogen), 2.5 µl of the template DNA, and sterile nuclease-free water to a final volume of 25 µl. The Mastercycler (Eppendorf®, Germany) with a heating lid was used for amplification.

3.9.3 Amplification conditions

The 25µl of reaction mixture was cycled in a Mastercycler (Eppendorf®, Germany) through the following temperature profile: an initial denaturation step at 94°C for 1 min; 45 cycles of 94°C for 1 min, 36°C for 1 min, and 72°C for 2 min; and a final

elongation step at 72°C for 5 min. The PCR products were held at 4°C until electrophoreses were performed.

3.9.4 Gel electrophoresis and interpretation

The amplified DNA fragments were electrophoresed in a horizontal 1% (w/v) agarose gels (Molecular grade (low EEO), Whitehead Scientific (Pty) Ltd) and stained with ethidium bromide (Promega, Madison, USA) 0.005% (v/v). For every sample. 5µl of the amplicon was charged into the respective wells of the agarose gel bathed in 1X TBE buffer made by 1:10 dilution of 10X TBE buffer (0.45M Tris, 0.44M Boric acid and 0.01M EDTA) (SIGMA®, Sigma Chemical Co., St Louis, USA).

Three microlitre of 2 log DNA ladder (BioLabs® Inc, New England) mixed with loading dye as per manufacturer's instructions was used as a size marker for the PCR products. The mixture was charged into the agarose wells in every round of electrophoresis. The agarose gels were electrophoresed at 60 volts for 90 minutes and photographed by using a computerized image capturing machine, Kodak 4000®. Gel images were imported to computer software, BioNumerics version 4.61 (Applied Maths). The images were analyzed and dendrograms generated.

3.10 Statistical analysis

Data collected by sample collection forms (biodata and risk factors associated with human infection) were stored in a Microsoft Office Access 2003 database and

analyzed using Epi Info 6 software (Coulombier *et al.*, 2001). Comparison of dichotomous variables was done by using Chi-square (χ^2) test at a critical probability of 0.05 and 95% confidence interval. The banding patterns of the isolates were analyzed using BioNumerics version 4.61 (Applied Maths, Belgium) whereby pairwise comparisons using the Dice similarity coefficient, and dendrograms were created using the unweighted pair group method using a geometric average (UPGMA). For the whole dataset, the most appropriate optimisation and position tolerance settings, as determined by the software, were 0 % and 1 %, respectively. For standardization purposes, the 2 log DNA ladder was run in all first and last wells of every row in the gel. Five of 114 *C. jejuni* (P216, P104, H0, H21 and H14) and four of 38 *C. coli* (P243, C309, C320, and C204) were run in duplicates for consistency.

3.11 Ethical clearance

The ethical clearance was applied for and granted before human sampling by the National Institute for Medical Research (NIMR) in Tanzania (No. NIMR/HQ/R.8a/Vol IX/303). The consent was sought from parents/guardians of the children bringing them to the health facilities and only children whose parents/guardians willingly accepted to participate were enrolled. After sample processing, confidentiality of the results was highly observed. In addition, feedback was given to the responsible medical staff on regular basis to assist routine diagnosis and further follow up.

CHAPTER FOUR

4.0 RESULTS

4.1 Thermophilic campylobacter infection in children

A total of 268 stool samples from children less than five years (54% males and 46% females) from different localities (Fig. 3) within Morogoro municipality were analyzed. Prevalence of thermophilic campylobacter infection in the children less than five years was 19.0%. Higher frequency of isolation was observed in males (66.7%) than females (33.3%) and the difference was statistically significant ($p<0.05$) as shown in Table 2. *Campylobacter jejuni* (78.4%) was the most commonly recovered isolate ($p<0.05$) than *C. coli* (19.6%). About 2% was unidentified isolate and *C. lari* was not isolated at all. Considering places of origin (Fig. 3), higher frequency of isolation was observed in children from Sabasaba (40.0%), Modecco (38.5%) and Kiwanja cha ndege (K/ndege) (28.0%). Children from Bigwa, Mafiga, Kihonda and Kilakala had relatively lower frequencies of isolation (<12.0% each). Frequencies of isolation of 21.6% and 12.8% were obtained in non-diarrhoeic and diarrhoeic children, respectively but the difference was not statistically significant ($p>0.05$).

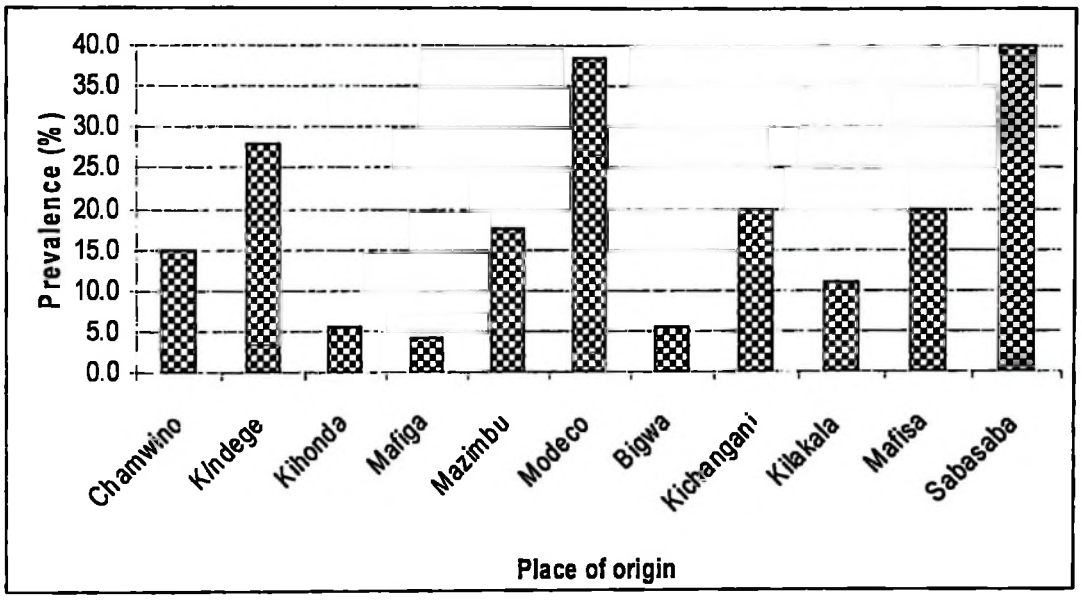

Figure 3. Frequency distribution of thermophilic campylobacter in children by source

Table 2. Risk factors for campylobacter infection in children

Risk factor	Group	Number of children (n)	Frequency of isolation (%)	P-value	Comment*
Age (months)	3-13	51	13.7		
	13-24	89	21.4		
	25-36	54	22.2	p>0.05	NS
	37-48	41	19.5		
	49-60	33	15.2		
Sex	Female	123	13.8	p<0.05	S
	Male	145	23.5		
Keeping chickens	At home	84	11.9		
	At neighbourhood	58	24.1	p>0.05	NS
	Not at all	126	21.4		
Keeping other animals	Kept	88	17.1	p>0.05	NS
	Not kept	180	20.5		
Drinking water treatment	Boiled/treated	161	16.7	p>0.05	NS
	Unboiled/untreated	107	22.4		
Diarrhoea	Diarrhoeic	78	12.8	p>0.05	NS
	Non-diarrhoeic	190	21.6		

* S = statistically significant and NS = statistically not significant

4.2 Thermophilic campylobacter infection in chickens

A total of 419 chicken cloacal swabs were collected from 22 chicken flocks located in different areas within the municipality (Table 3). Thirty seven percent (37.2%) of all samples were obtained from broilers, 42.0% layers; 15.3% indigenous and 5.5% cockerels. The prevalence of thermophilic campylobacter infection in chickens was 42.5% with frequency of isolation varying from 0% in one broiler flock to 100% in one of the indigenous chicken flocks. As shown in Table 3, *Campylobacter jejuni* was predominant isolated species (87%) in most localities, *C. coli* 13% and *C. lari* was not isolated. While *C. coli* were recovered from only four areas, *C. jejuni* was isolated from all localities except Kichangani.

Table 3. Frequency of isolation of thermophilic campylobacter species by area

Place	Number of chickens (n)	Frequency of isolation* (%)	
		C. jejuni	*C. coli*
Miembeni	74	79.7	1.4
Kilakala	84	29.8	21.4
Kihonda	75	21.3	2.7
Rocky Garden	15	100.0	0.0
Morogoro Central Market	17	82.4	0.0
Kididimo	59	20.0	0.0
Mazimbu	39	30.8	0.0
SUA FVM	37	2.7	5.4
Kichangani	19	0.0	0.0
Overall	419	87.1	12.9

*The frequency of isolation is based at individual bird level

As depicted in Table 4, higher isolation frequency ($p<0.05$) was observed in indigenous chickens (75.0%) than cockerels (52.2%) and broilers (50.0%). Layers had the least isolation frequency of 22.7%, which was statistically significant as compared to other chicken types ($p<0.05$). With respect to flock size, larger and small sized chicken farms/flocks had higher frequency of isolation ($p<0.05$) than medium one. Chickens kept by farmers who did not keep other species of animals at home had lower isolation frequency ($p<0.05$) than chickens kept by farmers who also kept other animals. Chickens aged 15-19 weeks had more isolation frequency (66.7%) than other age groups while those aged 5-9 weeks had the least isolation

frequency (14.6%). Mean chicken flock sizes were 1 010 chickens for broilers, 205 indigenous, 76 cockerels and 715 layers. Basing on farmers records, age in weeks (Mean±SD) of commercial chickens were: broilers 12.4±6, cockerels 65.6±2 and layers 50.4±24. The age of 49 birds out of 64 indigenous local chickens could not be determined, hence all indigenous chickens were judged and recorded as either adults or chicks; only the adults were involved in this study.

Table 4. Risk factors for campylobacter infection and isolation frequency in chickens

Risk factor	Group	Number of chickens (n)	Frequency (%)	P-value	Comment*
Chicken types	Broilers	156	50.0	p<0.05	S
	Cockerels	23	52.2		
	Indigenous	64	75.0		
	Layers	176	22.7		
Flock size	Small	199	37.7	P<0.05	S
	Medium	19	0.0		
	Large	201	51.2		
Keeping other animals	Kept	242	31.4	P<0.05	S
	Not kept	177	57.6		
Age** (weeks)	3-4	11	27.3	P<0.05	S
	5-9	48	14.6		
	10-14	28	21.4		
	15-19	93	66.7		
	20+	190	29.5		

*S = statistically significant (p<0.05)

** 49 indigenous chickens were excluded as their age could not be determined due to lack of proper records.

4.3 Genetic relatedness among thermophilic campylobacter isolates

Randomly selected representative isolates of *C. jejuni* (74 from chickens and 40 humans, total 114) and *C. coli* isolates (28 from chickens and 10 humans, total 38) were analyzed by RAPD PCR technique using OPA 11 primer (5'-CAA TCG CCG T-3'). All the isolates were successfully typed, their banding patterns (some of which are shown in Fig. 4) analyzed and the results displayed in the form of dendrograms (Fig. 5 and 6). However, one isolate (P29) yielded no bands and was not included in the analysis.

The results revealed 28 clusters of *C. jejuni* isolates at 85% homology, which could not be matched to their respective localities of origin. The number of individuals per cluster ranged from one to seven. *Campylobacter jejuni* and *C. coli* isolates were genetically diverse and seemed not to be specific to either of the hosts. Some *C. jejuni* (6.1%) and *C. coli* (5.3%) from humans and chickens showed similar banding patterns, implying that probably they were shared between humans and chickens. For *C. jejuni*, these isolates with their place of origin in brackets were: C156 (Mazimbu) and H54 (Chamwino); H0 (Urban) and P27 (SUA); H16 (Kiwanja cha ndege), H91 (Sabasaba) and C180 (Miembeni).

With respect to *C. coli* isolates, nine clusters were observed with one to nine individuals per cluster at 70% similarity level. The clustering of these isolates did not reflect geographical relationship as places of origin were randomly distributed between the clusters. Two *C. coli* isolates (5.3%) namely P241 from Kibwaya and

H'193 from Sabasaba showed similar banding patterns at 100% similarity level. Also, two human isolates (H'255 from Kilakala and H'30 from Chamwino) had also 100% similar banding patterns. Some *C. jejuni* isolates from same flocks (C191, C178, C164, C174 *et al.* from Miembeni) showed 100% similar banding patterns. Similar observation was made with *C. coli* isolates from another flock at Kilakala (C320 and 315). Similar situation was also observed with few *C. jejuni* isolates from humans though, surprisingly, these human isolates were from different areas. For example, H13 and H91 were recovered from Chamwino and Sabasaba, respectively while H'255 originated from Kilakala and H'30 was recovered from Chamwino.

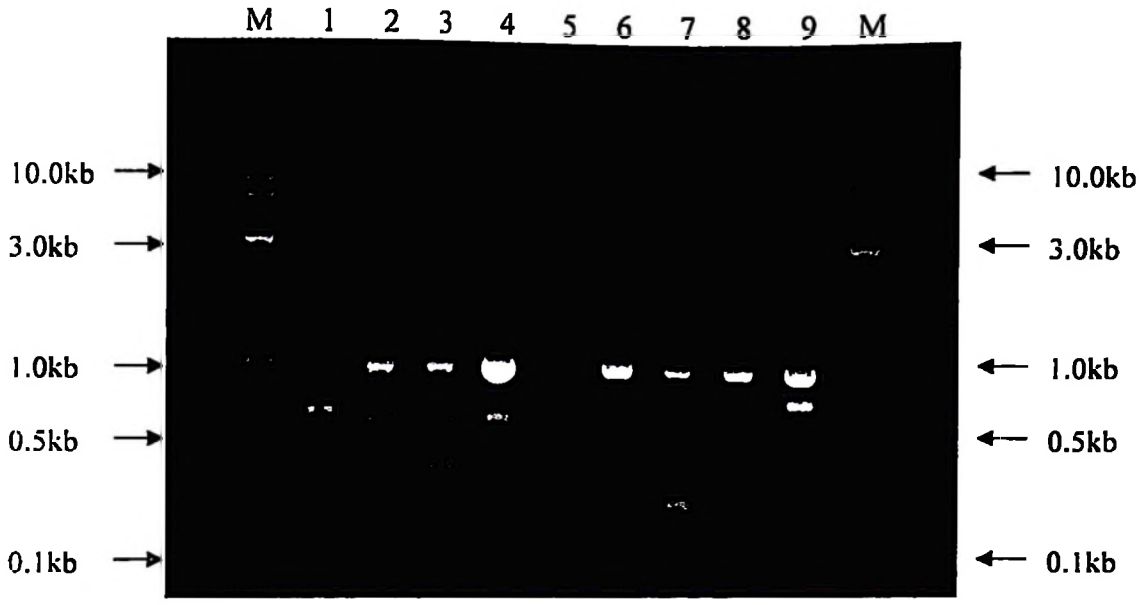

Figure 4. Gel electrophoresis of RAPD-PCR products using OPA 11 primer. Upper lanes: M is a 2 log DNA ladder, lanes 1 and 7 are *C. jejuni* from humans; 2, 3, 5, 6 and 9 are *C. jejuni* from indigenous local chickens; 4 is *C. coli* from human and lane 8 is *C. coli* from indigenous local chicken.

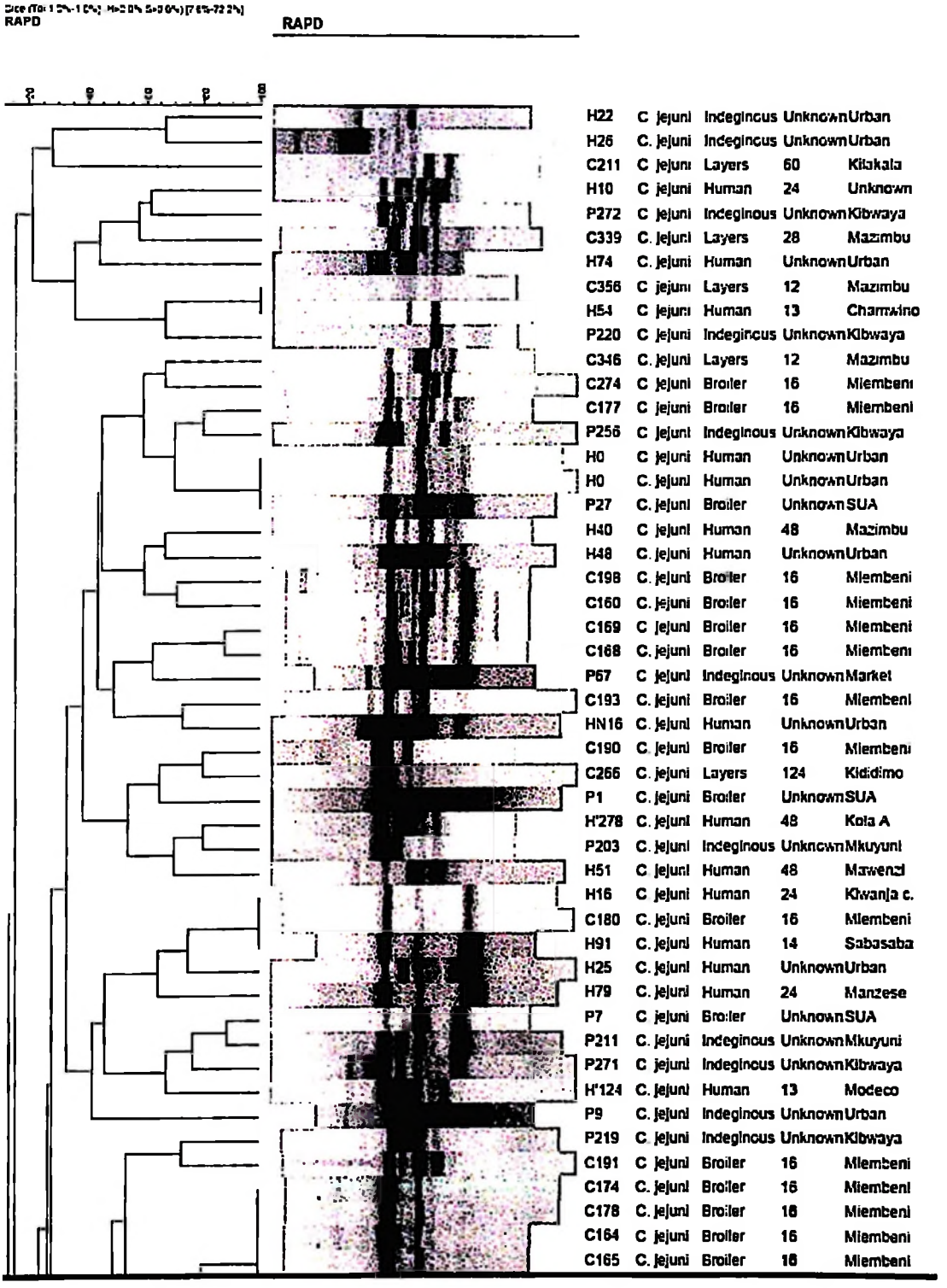

Figure 5. Part of phylogenetic dendrogram showing genetic relatedness among some *C. jejuni* isolates from humans and chickens.

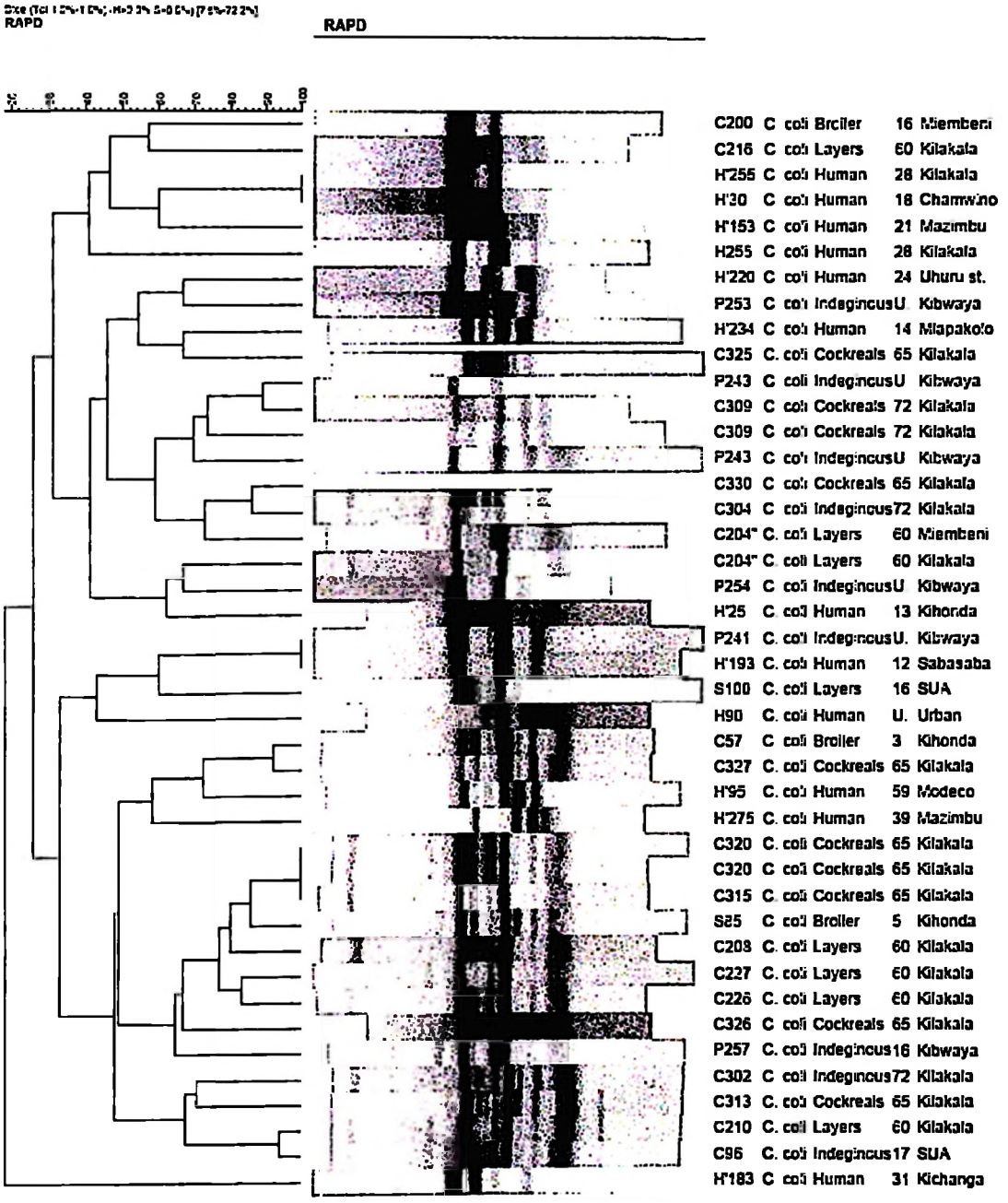

Figure 6. Phylogenetic dendrogram showing genetic relatedness among *C. coli* isolates from humans and chickens.

Therefore, RAPD-PCR technique with OPA 11 primer has shown good ability to discriminate campylobacter isolates from human and chicken hosts. Surprisingly, however, four *C. jejuni* isolates seemed to be 100% similar to four *C. coli*. *Campylobacter jejuni* C191 and C183 were similar to *C. coli* H'234 and S100, respectively. Likewise, other four isolates made up of *C. jejuni* C163, C186 and *C. coli* H'255 and H'30 were 100% similar. These were equivalent to 5% of all 152 *C. jejuni* and *C. coli* isolates from humans and chickens.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Thermophilic campylobacter infection in children

Diarrhoeal diseases cause high mortalities especially in children under the age of five, older people above 65 years and immunocompromised individuals in Tanzania (Ministry of Health, 1997). In most instances, campylobacter enteritis is observed in children below five years (Trachoo, 2003) and the young as compared to adults (Mdegela *et al.*, 2006). The prevalence of thermophilic campylobacter infection of 19% observed in this study is comparable to 18% reported in children less than five years in Dar es salaam (Lindblom *et al.*, 1995b). The current prevalence (19%) is slightly higher than 16% and 15.4% reported earlier by Nonga (2005) and Mdegela *et al.* (2006) in children under 15 years, respectively. Comparable prevalences of campylobacter infections in children less than five years have also been reported elsewhere in the developing countries. These prevalences were 15.25% in West Bengal (Rani *et al.*, 2004), 16.5% in Nigeria (Coker and Adefeso, 1994), 17.4% in Bangladesh (Albert *et al.*, 1999), 18% in Lawalpindi and Islamabad (Ali *et al.*, 2003).

With respect to higher prevalence of *C. jejuni* than *C. coli*, similar observations were also reported in different countries (Nachamkin and Blaser, 2000; Trachoo, 2003; Nonga, 2005; Mdegela *et al.*, 2006). Therefore, findings that *C. jejuni* is the leading cause of human campylobacter infection (78.4%) followed by *C. coli* (19.6%) and

non-isolation of *C. lari* have consistently been reported. Higher prevalence of *C. jejuni* than *C. coli* can be explained by different mechanisms in the pathogenesis by which the two pathogens are handled within the host cells. *Campylobacter coli* are in most cases phagocytosed and killed by peritoneal macrophages than *C. jejuni* (Banfi *et al.*, 1986). Though it has been demonstrated *in vitro*, this could be happening *in situ* in the human cells. This might explain why infections due to *C. coli* are not as severe as those caused by *C. jejuni*. In addition, *C. coli* infections have less often been associated with bloody diarrhea and symptomatic disease as compared to *C. jejuni* infections (Taylor *et al.*, 1998).

Nutritional status of children is also an important factor for morbidity and mortality in children. Undernutrition is one of the major public health concerns in developing countries, where it represents both a cause and a manifestation of poverty (Madise *et al.*, 1999). Low family income, poor education, poor environment and housing, and inadequate access to foods, safe water and health care services are attributes of poverty. Malnourished children are most easily infected by thermophilic campylobacter species and other pathogens than well-nourished ones.

In other studies, keeping of animals in closer proximity with humans was reported as a risk factor to acquire zoonotic diseases. In a study conducted in West Bengal by Rani *et al.* (2004), children from families that kept poultry and cattle had significantly higher prevalence of campylobacter infections (27.2%) than their counterparts without those animals (3.4%). Toddlers frequently come into contact

with poultry faeces and have an average of almost 4 faeces-to-mouth episodes in 12-hours (Marquis *et al.*, 1990). The fact that culture of poultry faeces yielded viable *C. jejuni* for up to 48 hours after deposition suggests existence of high risk of campylobacter transmission in environments where there may be frequent human-animal contact (Marquis *et al.*, 1990). Presence of animals coupled with poor hygiene and interaction of humans with animals is a risk factor for campylobacter infection rather than a mere ownership of animals. This complex interaction substantiates observation of the current study that keeping chickens or other animals had no influence on children infection.

The significantly higher isolation rate of campylobacter species from males than females supports findings by other studies (Coker and Adefenso, 1994; Ali *et al.*, 2003). In those studies, reported male to female risk ratios of and 1.7:1 and 2:1 were reported. Other previous studies reported no sex preference in campylobacter infections in humans (Nonga, 2005; Mdegela, 2006). Although the actual cause of higher frequency of isolations in males than females is yet to be established, sex predilection due to hormonal influence and different levels of exposure to sources of infection could possibly be contributing factors.

Diarrhoea is one of main clinical signs of campylobacter infection. The frequency of isolation was not significantly different ($p>0.05$) between non-diarrhoeic children (21.6%) and the diarrhoeic ones (12.8%). Similar trend was observed by Rani *et al.* (2004), who reported higher prevalence in diarrhoeic children (20%) as compared to

apparently healthy non-diarrhoeic ones (11.8%). Higher prevalence of infection in non-diarrhoeic children indicates presence of asymptomatic carriers in children population. Endemic infection can be implicated in a situation whereby children are infected at early ages and experience asymptomatic infection later on as they grow up (Ali *et al.*, 2003). Regardless of the child's age, first exposure to infective levels of campylobacter species was reported to be pathogenic but a subsequent infection after six months of age was not (Ali *et al.*, 2003). The infected children can excrete campylobacter species in faeces for long period of up to one month after diarrhoeal episodes (Rao *et al.*, 2001).

5.2 Thermophilic campylobacter infection in chickens

Thermophilic campylobacter species can be found in intestines of a wide range of warm-blooded animals. *Campylobacter jejuni* is reported to behave as a commensal to avian hosts although it is pathogenic to humans (Saleha, 2004; Bryne *et al.*, 2007). The current study revealed prevalence of thermophilic campylobacter infection of 42.5% in chickens. This prevalence is by far lower than 70% earlier reported by Nonga (2005) in Morogoro.

Higher frequency of isolation of these enteric bacteria was observed in indigenous local chickens than broilers, layers and cockerels ($p<0.05$). This finding is contrary to other reports that revealed comparable frequencies of isolation between indigenous local chickens and other types (Nonga, 2005; Mdegela *et al.*, 2006). Several studies

portrayed the role of poultry, especially chickens in transmitting campylobacter infections to humans. Majority of chickens kept in Tanzania are indigenous and they are managed under the free-range system. The chickens move freely and spread their droppings around homesteads, which poses more public health concerns. While feeding inside their houses or while scavenging, chickens might pick these bacteria from: inadequately cleaned and disinfected poultry houses; polluted water; reuse of old litter; feed; rodents and free-flying wild and domestic birds; lesser mealworm; pests and insects like houseflies and darkling beetles (Shane *et al.*, 1985; Genigeorgis *et al.*, 1986; Kazwala *et al.*, 1990; van de Giessen *et al.*, 1993; Saleha *et al.*, 1998).

According to farmers' practices observed in the current study area, poor biosecurity, types of chickens and poor hygiene might have contributed to different isolation frequencies observed. With populations ranging from 1-199, 200-299 and 300-7 000, chicken farms/flocks were respectively regarded as small, medium and large. Poor biosecurity coupled with poor cleanliness of the chicken houses might contribute to higher frequency of isolation in large flocks. As the number of chickens increases, demands for hygiene/ cleanliness and biosecurity become bigger. Large flocks were not cleaned effectively and their biosecurity was poorer as compared to small and medium flocks; hence, the risk for infection increased. On the other side, many smaller flocks contained indigenous chickens, which were found to have higher isolation frequency of campylobacter species than broilers, layers, and cockerels. However, the medium flocks seemed to have conveniently manageable size and optimum hygiene/ cleanliness and biosecurity levels; hence, lower risk for infections.

The isolation of thermophilic campylobacter species was observed in all age groups of chickens under this study. Higher frequency of isolation was observed in 15-19 week age group as compared to other groups. Since campylobacter species are commensal to gastrointestinal track of chickens, the older the chicken age the larger the load of campylobacter species. Loads of campylobacter species were found to increase with age in broilers and, as the age increases, campylobacter carriage levels of up to 1.2 x 10^7c.f.u/g have been reported in poultry (Kazwala *et al.*, 1988; Nonga, 2005; Saleha. *et al.*, 1998). Frequency of isolation in chickens of four weeks of age was 64.3% but the levels shot up significantly to 93% at the age of 39 weeks (Nonga, 2005). These scientists concluded that older chicken age is associated with prolonged exposure to thermophilic campylobacter species from disparate sources.

The observation that keeping other animals at a household where chickens were kept was a risk factor for infection but in favour of concurrent keeping is surprising. Chicken kept by farmers who also kept other animal species at home were less infected than those owned by farmers who did not keep other animals ($p<0.05$). A lesson from a study on transmission between humans and chickens conducted elsewhere (Marquis *et al.*, 1990) might explain this observation. In that study, it was concluded that keeping other animals has to couple with poor hygiene and interspecies interactions in order for it to act as a risk factor for infection. Otherwise, a mere keeping of animals could not influence outcomes of infection. Transmission

of campylobacter species between chickens and other animals must be preceded by direct or indirect interaction of chickens and those animals.

Culture and isolation techniques such as the use of cloacal swabs for sampling may have influenced results in this study. Sampling technique that used cloacal swabs was less sensitive as compared to the one that used intestinal contents (Nonga, 2005). Caeca are the best colonization sites of thermophilic campylobacter species in the gastrointestinal tract (Kazwala *et al.*, 1992; Nonga, 2005). At this certain stage, these bacteria can only be found in caeca and not elsewhere at levels that are too low to detect in faeces (Kazwala *et al.*, 1992). This phenomenon could contribute to lower prevalence of campylobacter infections observed in chickens in the current study as compared to previous studies. The current study was conducted when there was a ban on importation of chickens and eggs due to threat of introducing avian influenza in Tanzania. In this period, chickens were scarcely available and the demand was high making farmers keener in improving management practices. Improvement of management probably helped to lower exposure potential and consequently lowering the observed prevalence of infection.

Indigenous local chickens are usually kept under extensive management in many parts of Tanzania as opposed to commercial chickens (broilers, layers, and cockerels) which are intensively kept. The indigenous local chickens were probably more exposed to and interacted with some risk factors either directly or indirectly. These risk factors include: vermin, rodents, kitchen wastes, contaminated water and

environments, and other infected animals (Saleha *et al.*, 1998). Insecure entrance to chicken bans or sheds by people, especially those dealing closely with these chickens, could have had transmitted pathogens to chickens. This could happen in various situations, for example, while serving food and water, handling them for various reasons. Other sources of infection could be newly introduced chickens from other farms or areas, specifically if the chickens in question had been infected.

Free-range indigenous and commercial chickens may interact with other animal species in daily life. Some other animal species rather than chickens reported to carry and spread thermophilic campylobacter species include dogs, cats, goats, pigs and rats (Kasrazadeh and Genigeorgis, 1987; Franco, 1989; Skirrow, 1991; Raji, 2000). Some wild birds including seagulls, crows, blue magpies, grey starlings and sparrow and even insects like housefly also carry and spread campylobacter species (Rosef *et al.*, 1981; Kapperud and Rosef, 1983; Shane *et al.*, 1985; Ito *et al.*, 1988; Mdegela *et al.*, 2006). Farmers in urban and peri-urban areas of Morogoro municipality commonly keep multispecies of domestic animals. On the other hand, some domestic and wild animals and birds scavenge for food in dumping places, where there is also higher fly activity. The scavenging is encouraged by improper disposal and/or delayed collection of wastes, especially kitchen wastes and food left over from hotels, kiosks or food vendors. Interaction of chickens and some of scavenging animals could transmit thermophilic campylobacter species between these animals and chickens.

As in humans, isolation of *C. jejuni* was significantly higher than *C. coli* and *C. lari* was not isolated. This observation is congruent with other findings (Engvall *et al.*, 1986; Kazwala, 1990; Saleha, *et al.*, 1998) where *C. jejuni* were more prevalent than *C. coli* and *C. lari* isolates from disparate hosts. *Campylobacter jejuni* was wide spread as it was isolated from all localities except Kichangani while *C. coli* were recovered from four areas (Kilakala, SUA FVM, Kihonda and Miembeni). Almost all the flocks were located nearby residential houses and people including children had unrestricted access to the chicken houses except at SUA FVM. Unrestricted access to chicken houses increases risk of transmission of thermophilic campylobacter species between chickens and humans.

Alarmingly, higher isolation of *C. jejuni* was observed in live chickens sold at Morogoro central market. A poor slaughter place was very closer to piled cages full of live chickens and slaughtering was done to cater for customers' demands. Chickens shed large numbers of campylobacter species and poor evisceration can easily contaminate chicken carcasses, surface water and environments (Saleha *et al.*, 1998). Under these situations, spread of campylobacter infections amongst chickens and from chickens to humans is most probable. Insecure slaughtering of chickens still continues at Morogoro Central Market up to this time (January, 2009). Unless adequate measures for improvement are taken, there is high risk to public health.

5.3 Genetic relatedness among human and chicken campylobacter isolates

Highly diverse banding patterns were revealed by the current study, implying that there were several strains of *C. jejuni* and *C. coli* prevailing in human and chicken populations. Genetic diversity is one of mechanisms that help pathogens including campylobacter species adapt to adverse environments and renders them with ability to colonize multiple animal hosts. This phenomenon is important in perpetuating campylobacter infections among human and animal populations. Various mechanisms can generate genomic diversity that helps pathogens to escape hosts' immunological responses. Genomic rearrangement and horizontal gene transfer are among these adaptive mechanisms that could have played a role in resulting to thermophilic campylobacter diversity observed in this study (Wassenaar *et al.*, 2000). Alteration in genetic make up of microorganisms could also be a result of mutations, which may occur spontaneously in normal life.

Some similar banding patterns at 100% similarity level were observed among both *C. jejuni* (6.1%) and *C. coli* isolates (5.3%) from humans and chickens. This finding increases probability that the strains were shared between humans and chickens. Probably one host acted as a reservoir of infection to the other, justifying existence of zoonotic campylobacter infections in humans and chicken populations in Morogoro municipality. Previous studies conducted in different areas in the world reported similarity among thermophilic campylobacter isolates from human and chickens (Nadeau *et al.*, 2002; Workman *et al.*, 2005; Rozynek *et al.*, 2006). In Poland, cluster analysis of RAPD patterns from 115 *Campylobacter* isolates from chickens and 80

from children revealed that six human *C. coli* isolates were identical to chicken isolates (Rozynek *et al.*, 2006). Workman *et al.* (2005) in Barbados reported that the OPA 11 fingerprints of one human *C. jejuni* and five human *C. coli* isolates were identical to one *C. jejuni* and five *C. coli* from chicken meat, respectively. However, these similar strains were not identical upon further discrimination by another primer. In Canada, Nadeau *et al.* (2002) reported that macrorestriction profiles of approximately 20% of human campylobacter isolates were genetically related to genotypes found in poultry. This finding showed that chickens were sources of human infections.

Unexpectedly, some *C. jejuni* isolates had banding patterns that were 100% similar to those of *C. coli*. These isolates had previously been classified into different species by biochemical tests. This finding is in line with other studies (Steinbrueckner *et al.*, 1999; Nonga, 2005; Mdegela *et al.*, 2006). Steinbrueckner *et al.* (1999) reported that biochemical tests misclassified some campylobacter strains that were later on correctly diagnosed by the PCR. About 26% of 243 *C. jejuni* isolates that had previously been identified as positive by biochemical tests later on turned to be negative on PCR (Nonga, 2005). Due to its high sensitivity, PCR can be used to identify isolates into their respective species more accurately than biochemical tests. Considering bottlenecks of conventional methods, it was recommended that preliminary identification by biochemical tests should be subjected to confirmation by PCR (Mdegela *et al.*, 2006). The findings of this study complement this recommendation and suggest that PCR technique be mainstreamed in the laboratories

dealing with campylobacter in order to iron out anomalies of biochemical characterization.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

For the first time, the current study has shown existence of zoonotic transmission between humans and chickens in Morogoro municipality. Although *C. jejuni* and *C. coli* isolates were highly diverse, the RAPD profile of 6.1% of *C. jejuni* and 5.3% of *C. coli* from humans and chickens were genetically similar. This is an adequate proof that some thermophilic campylobacter isolates were shared between human and chicken populations. Therefore, chickens should be given special attention as possible source(s) of thermophilic campylobacter infections to humans.

This study has also shown that thermophilic campylobacter infections are prevalent both in children less than five years and chickens and *C. jejuni* is the leading cause followed by *C. coli*. *Campylobacter lari* was neither isolated from humans nor chickens; hence, it is a rare species. With respect to human infections, male children had higher frequency of isolation than females. On the other hand, risk factors influencing campylobacter infections in chickens were: chicken types, flock size and keeping other animal species together with chickens. In transmitting thermophilic campylobacter species between human and animal hosts or among animal species, interspecies interaction is important than a mere co-existence of multispecies.

6.2 Recommendations

By the fact that zoonotic transmission has been confirmed by RAPD genotyping, more diversified genomic and phenotypic studies are needed to explore further genomic diversity among isolates from disparate hosts. Domestic animal species that are at a closer proximity with humans in day-to-day life should be given priority by those studies. Besides chickens, the role of other animal species in transmitting campylobacter species to humans should be closely examined. In view of the fact that only few thermophilic campylobacter isolates from humans and chickens were found to have same type of RAPD patterns, further studies using different genotyping techniques are required to analyze more isolates and determine their genetic relatedness. The recommended studies may also explore which other species among domestic and wild animals act as major reservoir(s) for human infections in Tanzania.

Considering higher prevalence of infections both in humans and chickens and the existing risk factors, personal and environmental hygiene are strongly recommended. Hygiene will help lowering possibilities of spreading campylobacter species between and among humans and chickens. Hygiene has to be keenly observed by all stakeholders at different levels of food chains from production to consumption. All key stakeholders are hereby urged to play their parts duly in safeguarding public health not only in Morogoro municipality but also in other areas from which foods of animal origin, especially chickens are supplied.

Lastly, children below five years should be restricted from interacting with chickens. These children should not be allowed in chicken houses. However, if this is unavoidable, their hands have to be regularly cleaned before and after eating. Homesteads together with their surroundings should be regularly cleaned to reduce possibilities of children coming into contact with chicken droppings, which are sources of infection. After slaughter, preparation of raw chicken/poultry meat has to be done hygienically and thorough cooking is highly recommended. Chickens and poultry meat should be separated from other ready-to-eat foods to minimize chances of cross-contamination.

7.0 REFERENCES

Aarestrup, F. M. and Wagener, H. C. (1999). The effects of antibiotic usage in food animals on the development of antimicrobial resistance of importance for human in Campylobacter and *Escherischia coli. Microbes and Infection* 1: 639-644.

Acik, M. N. and Cetinkaya, B. (2006). Heterogeneity of *Campylobacter jejuni* and *Campylobacter coli* strains from healthy sheep. *Veterinary Microbiology* 115: 370–375.

Adetunji, J. (2000). Trends in under-5 mortality rates and HIV/AIDS epidemic. *Bulletin of the World Health Organization* 78: 1200 -1206.

Albert, M. J., Faruque, A. S., Faruqe, S. M., Sack, R. B., Mahalanabis, D. (1999). Case control study of enteropathogens associated with childhood diarrhoea in Dhaka, Bangladesh. *Journal of Clinical microbiology* 37: 345-364.

Ali, A. M., Qureshi, A. H., Rafi, S., Roshan, E., Khan, I., Malik, A. M. and Shahid, S. A. (2003). Frequency of *Campylobacter jejuni* in Diarrhoea/Dysentery in Children in Rawalpindi and Islamabad. *Journal of Pakistan Medical Association* 53 (11): 517-520.

Almashat, R. R. and Taylor, D. J. (1980). Campylobacter spp in enteric lesions in cattle. *Veterinary Record* 107: 31-34.

Altekruse, S. F., Stern, N. J., Fields, P. I. and Swedlow, D. L. (1999). *Campylobacter jejuni* an emerging food borne pathogen. *Emerging Infectious Diseases* 5: 28-35.

Altekruse, S. F. and Tollefson, L. K. (2003). Human campylobacteriosis: a challenge for the veterinary profession. American Veterinary Medical Association [http:// www.avma.org/reference/zoonosis/zncampylobacteriosis.asp] site visited on 8/6/2007.

Bacon, D. J., Alm, R. A., Burr, D. H, Hu, L., Kopecko, D. J., Ewing, C. P., Trust, T. J., Guerry, P. (2000). Involvement of a plasmid in virulence of *Campylobacter jejuni* 81–176. *Infection and Immunity* 68: 4384–4390.

Bacon, D. J., Alm, R. A., Hu, L., Hickey, T. E., Ewing, C. P., Batchelor, R. A., Trust, T. J. and Guerry, P. (2002). DNA sequence and mutational analyses of the pVir plasmid of *Campylobacter jejuni* 81–176. *Infection and Immunity* 70: 6242–6250.

Bacon, D. J., Szymanski, C. M., Burr, D. H., Silver, R. P., Alm, R. A. and Guerry, P. (2001). A phase-variable capsule is involved in virulence of *Campylobacter jejuni* 81–176. *Molecular Microbiology* 40: 769–777.

Banfi, E., Cinco, M. and Zabucchi, G. (1986). Phagocytosis of *Campylobacter jejuni* and *C. coli* by peritoneal macrophages. *Journal of General Microbiology* 132: 2409–2412.

Beery, J. T, Hugadal, M. B. and Doyle, M. P. (1988). Colonization of gatrointestinal tract of chicks by *Campylobacter jejuni*. *Applied and Environmental Microbiology* 84: 2365-2370.

Bhadra, R. K., Lior H., Misra, S. K., Pal, S. C. and Nair, G. B. (1989). Serotypes and biotypes of *Campylobacter jejuni* and *Escherischia coli* in Calcutta. *Indian Journal of Medical Research* 89: 225-228

Biswas, D., Itoh, K. and Sasakawa, C. (2000). Uptake pathways of clinical and healthy animal isolates of *Campylobacter jejuni* into INT-407 cells. *FEMS Immunology and Medical Microbiology* 29: 203–211.

Blaser, M. J. and Reller, L. B. (1981). Campylobacter enteritis. *National England Journal of Medicine* 305: 1444-1452.

Blaser, M. J. (1997). Epidemiologic and clinical features of *Campylobacter jejuni* infections. *Journal of Infectious Diseases* 176: 103-105.

Bourke, B. (2002). *Campylobacter* infection: small bowel and colon. *Current Opinions in Gastroenterology* 18: 4–9.

Byrne, C. M., Clyne M. and Bourke, B. (2007). *Campylobacter jejuni* adhere to and invade chicken intestinal epithelial cells in vitro. *Microbiology* 153: 561–569.

Butzler, J. P. and Oostrerom, J. (1991). *Campylobacter*: Pathogenicity and significance in foods. *International Journal of Food Microbiology* 12 (1): 1-8.

Carter, G.R. and Chengappa, M. M. (1991). *Essentials of Veterinary Bacteriology and Mycology, 4th edition*, Lea and Febiger, Philadelphia and London. 284pp.

Carvalho, A. C. T., Ruiz-Palacios, G. M., Cervantes, P. R., Cervantes, L. E., Jiang, X. and Pickering, L. K. (2001). Molecular Characterization of Invasive and Noninvasive *Campylobacter jejuni* and *Campylobacter coli* Isolates. *Journal of Clinical Microbiology* 39(4): 1353–1359.

Catteau, M. (1995). The genus Campylobacter. In: *Microbiological Control for Food and Agricultural Products. (Edited by Bourgeois, C. M. and Leveau, J. Y.),* VHC publishers Inc, New York. pp. 325-334.

Christensen, B., Sommer, H., Rosenquist, H. and Nielsen, N. (2001). Risk assessment on *Campylobacter jejuni* in chicken products. First edition. The Danish Veterinary and Food Administration, Institute of Food Safety and Toxicology Division of Microbiological Safety. [http://www.foedevarestyrelsen.dk/NR/rdonlyres/evf3g4j6ajare35t2psiehcdxjsnfni2tssdbmhqa5mfmd4uxuyp3iuixv6rpg2flkxm6iyhu3jd4ngeaixswsan4rh/rapport_jan2001.doc.] site visited on 10/6/2007.

Clark, A. G. and Bueschkens, D. H. (1988). Horizontal spread of human and poultry-derived strains of *Campylobacter jejuni* among broiler chicks held in incubators and shipping boxes. *Journal of Food Protection* 51: 438-441.

Coker, A. O. and Dosunmu-Ognubi, O. (1985). Gastroenteritis due to *Campylobacter jejuni* in Lagos, Nigeria. *Central African Journal of Medicine* 31: 72-4.

Coker, A. O. and Adefeso, A. O. (1994). The changing patterns of Campylobacter jejuni/coli in Lagos Nigeria after 10 years. *East African Medical Journal* 71: 437-40.

Coker, A. O., Isokpehi, R. D., Thomas, B. N., Amisu, K. O. and Obi, C. L. (2002). Human Campylobacteriosis in Developing Countries. *Emerging Infectious Diseases* 8 (3): 237-241.

Colegio, O. R., Griffin, T. J., Grindley, N. D. and Galan, J. E. (2001). *In vitro* transposition system for efficient generation of random mutants of *Campylobacter jejuni*. *Journal of Bacteriology* 183: 2384–2388.

Coulombier, D., Fagan, R., Hathcock, L. and Smith, C. (2001). Epi Info 6 version 6.04d. A word processing, database and statistical program for public health.

Centers for Disease Control and Prevention, Delaware, USA. [http://www.cdc.gov/epiinfo/downloads.htm] site visited on 3/2/2008.

Crushell, E., Harty, S., Sharif, F. and Bourke, B. (2004). Enteric *campylobacter*: purging its secrets? *Pediatric Research* 55: 3-12.

Dekeyser, P., Gossuin-Detran, M., Butzler, J. P. and Sternon. J. (1972). Acute enteritis due to related Vibrio: first positive stool cultures. *Journal of Infectious Diseases* 125: 390-392.

Ellis, A., Irwin, R., Hockin, J., Borckzyk, A., Woodward, D. and Johnson, W. (1995). Outbreak of Campylobacter infection among farm workers: An occupational hazard. *Canada Communicable Disease Report* 21: 153-156.

Ellsworth, D. L., Rittenhouse, K. D. and Honeycutt, R. L. (1993). Artifactual variation in randomly amplified polymorphic DNA banding patterns. *BioTechniques* 14: 214–217.

Elwell, C. A. and Dreyfus, L. A. (2000). DNase I homologous residues in CdtB are critical for cytolethal distending toxin-mediated cell cycle arrest. *Molecular Microbiology* 37: 952–963.

Engberg, J., Aarestrup, F. M., Taylor, D. E., Gerner-Smidt, P. and Nachamkin, I. (2001). Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: resistance mechanism and trends in human isolates. *Emerging Infectious Diseases* 7: 24-34.

Engvall, A., Bergovist, A., Sandstedt, K. and Danielsson-Tham, M. L. (1986). Colonization of broiler with Campylobacter in conventional broiler chicken flocks. *Acta Veterinaria Scandinavica* 27: 540-547.

Euzéby, J. P. (1997): List of Bacterial Names with Standing in Nomenclature. *International Journal of Systemic Bacteriology* 47: 590-592.

Franco, D. A. (1989). Campylobacteriosis: the complexity of control and prevention. *Journal of Environmental Health* 52: 88-92.

Fry, B. N., Feng, S., Chen Y. Y., Newell, D. G., Coloe, P. J. and Korolik, V. (2000). The *galE* gene of *Campylobacter jejuni* is involved in lipopolysaccharide synthesis and virulence. *Infection and Immunity* 68: 2594–2601.

Genigeorgis, C., Hassuneh, M. and Collins, P. (1986). *Campylobacter jejuni* infection in poultry farms and its effects on poultry meat during slaughtering. *Journal of Food Protection* 49: 895-903.

Grant, I. H., Richardson N. J. and Bokkenheuser, V. D. (1980). Broiler chickens as potential source of Campylobacter infection in humans. *Journal of Clinical Microbiology* 11: 508-510.

Hald, B. and Madsen, M. (1997). Health puppies and kittens as carriers of Campylobacter spp., with special reference to Campylobacter upsaliensis. *Journal of Clinical Microbiology* 35: 3351-3352.

Hansson, I. (2007). Bacteriological and Epidemiological Studies of *Campylobacter* spp. in Swedish Broilers. Dissertation for award of PhD Degree at Swedish University of Agricultural Sciences, Uppsala, Sweden. 56pp.

Hendrixson, D. R., Akerley, B. J. and DiRita, V. J. (2001). Transposon mutagenesis of *Campylobacter jejuni* identifies a bipartite energy taxis system required for motility. *Molecular Microbiology* 40: 214–224.

Hernandez, J., Fayos, A., Ferrus, M. A. and Owen, R. J. (1995). Random amplified polymorphic DNA fingerprinting of *Campylobacter jejuni* and *C. coli* isolated from human faeces, seawater and poultry products. *Research in Microbiology* 146: 685–696.

Hernandez, J., Fayos, A., Alonso, J. L. and Owen, R. J. (1996). Ribotypes and AP-PCR fingerprints of thermophilic campylobacters from marine recreational waters. *Journal of Applied Bacteriology* 80: 157–164.

Hickey, T. E., Baqar, S., Bourgeois, A. L., Ewing, C. P. and Guerry, P. (1999). *Campylobacter jejuni*-stimulated secretion of interleukin-8 by INT407 cells. *Infection and Immunity* 67: 88–93.

Hickey, T. E., McVeigh, A. L., Scott, D. A., Michielutti, R. E., Bixby, A., Carroll, S. A., Bourgeois, A. L. and Guerry, P. (2000). *Campylobacter jejuni* cytolethal distending toxin mediates release of interleukin-8 from intestinal epithelial cells. *Infection and Immunity* 68: 6535–6541.

Hu, L. and Kopecko, D. J. (1999): *Campylobacter jejuni* 81–176 associates with microtubules and dynein during invasion of human intestinal cells. *Infection and Immunity* 67: 4171–4182.

Ito, K., Kubokura, Y. Kaneko, K. Totake Y. and Ogawa, M. (1988). Occurrence of *Campylobacter jejuni* in free-living wild birds from Japan. *Journal of Wildlife Diseases* 24: 467-470.

Jacobs-Reitzma W. F., van de Giessen, A.W., Boler, N. M. and Malder, R. W. A. W. (1995). Epidemiology of Campylobacter spp. at two Dutch broiler farms. *Epidemiology and Infections* 114: 413-421.

Jin, S., Joe A., Lynett, J., Hani, E. K., Sherman, P. and Chan, V. L. (2001). JlpA, a novel surface-exposed lipoprotein specific to *Campylobacter jejuni*, mediates adherence to host epithelial cells. *Molecular Microbiology* 39: 1225–1236.

Jiwa, S. F. H., Mdegela, R. H. and Kazwala, R. R. (1997): Epidemiology of thermophilic Campylobacter species in apparently healthy domestic ducks kept as free range without veterinary inputs in Morogoro municipality. *Tanzania Veterinary Journal* 17: 73–81.

Jones, D. M. and Robinson, D.A. (1981). Occupational exposure to *Campylobacter jejuni* infection. *Lancet* I: 440-441.

Kapperud, G. and Rosef, O. (1983). Avian wildlife reservoir of Campylobacter fetus subsp. jejuni, Yersinia spp. and Salmonella spp. in Norway. *Applied Environmental Microbiology* 45(2): 375-380.

Karlyshev, A. V. and Wren, B. W. (2001). Detection and initial characterization of novel capsular polysaccharide among diverse *Campylobacter jejuni* strains using alcian blue dye. *Journal of Clinical Microbiology* 39: 279–284.

Kasrazadeh, M. and Genigeorgis, C. (1987). Origin and prevalence of Campylobacter jejuni in ducks at the farm and processing plant level. *Journal of Food Protection* 50: 321-326.

Kazwala, R. R. (1988). Studies on Origin, and Quantitative Distribution of Thermophilic Campylobacters at various stages of Poultry Production and Poultry Processing. Thesis for award of Master of Veterinary Medicine at the National University of Ireland, Ireland. 243pp.

Kazwala, R. R., Collins, J. D. and Hanna, J. (1992): Establishment and experimental spread of *Campylobacter jejuni* infections in young chickens. *Preventive Veterinary Medicine* 13: 19-26.

Kazwala, R. R., Collins, J. D., Hanna, J., Crion, R. A. P. and O'Mahony, H. (1990). Factors responsible for introduction and spread of *Campylobacter jejuni* in commercial poultry production. *Veterinary Record* 126: 305-306.

Ketley, J. M. (1997). Pathogenesis of enteric infection by campylobacter. *Microbiology* 143: 5-21.

Koenraad, P. M. F. J., Ayling, R. and Hazeleger, W. C. (1995). Speciation and subtyping of *Campylobacter* isolates from sewage plants and waste water from a connected poultry abattoir using molecular techniques. *Epidemiology and Infection* 115: 485-494.

Konkel, M. E., Kim B. J., Rivera-Amill, V. and Garvis, S. G. (1999). Bacterial secreted proteins are required for the internalization of *Campylobacter jejuni* into cultured mammalian cells. *Molecular Microbiology* 32: 691–701.

Konkel, M. E, , S. G., Tipton S. L, Anderson D. E. J. and Cieplak, W. J. (1997). Identification and molecular cloning of a gene encoding a fibronectin-binding protein (CadF) from *Campylobacter jejuni. Molecular Microbiology* 24: 953–963.

Kopecko, D. J., Hu, L. and Zaal, K. J. (2001): *Campylobacter jejuni*-microtubule-dependent invasion. *Trends in Microbiology* 9: 389–396.

Kusiluka, L. J. M., Karimuribo, E. D., Mdegela, R. H., Luoga, E. J., Munishi, P. K. T., Mlozi, M. R. S. and Kambarage, D. M. (2005). Prevalence and impact of

water-borne zoonotic pathogens in water, cattle and humans in selected villages in Dodoma Rural and Bagamoyo districts, Tanzania. *Physics and Chemistry of the Earth* 30: 818–825.

Laanbroek, H. J., Kingma, W. and Veldkamp, H. (1977). Isolation of an aspartate-fermenting, free-living *Campylobacter* species. *FEMS Microbiology Letters* 1: 99-102.

Lara-Tejero, M. and Galan, J. E. (2001). CdtA, CdtB, and CdtC form a tripartite complex that is required for cytolethal distending toxin activity. *Infection and Immunity* 69: 4358–4365.

Lastovica, A. J., Goddard, E. A. and Argent, A. C. (1997). Guillain-Barré syndrome in South Africa associated with *Campylobacter jejuni* O:41 strains. *Journal of Infectious Diseases* 176 (2): S139-143.

Lindblom, G. B., Ahren, C., Changalucha, J., Gabone, R., Kaijser, B., Nilsson L. A., Sjogren, E., Svennerholm, A. M. and Temu, M. (1995b): Campylobacter jejuni/coli and enterotoxigenic Escherichia coli (ETEC) in faeces from children and adults in Tanzania. *Scandinavian Journal of Infectious Diseases* 27(6): 589-593.

Lindblom, G. B., Sjogren, E., Westerberg, J. H. and Kaijser, B. (1995a). *Campylobacter upsaliensis*, *C. sputorum sputorum* and *C. concisus* as common causes of diarrhoea in Swedish children. *Scandinavian Journal of Infectious Diseases* 27: 187-188.

Lindblom, G. B., Sjorgren, E. and Kaijser, B. (1986). Natural campylobacter colonization in chickens raised under different environmental conditions. *Journal of Hygiene* 96: 385-391.

MacPherson, J. M., Eckstein, P. E., Scoles, G. J. and Gajadhar, A. A. (1993). Variability of the random amplified polymorphic DNA assay among thermal cycles, and effects of primer and DNA concentration. *Molecular and Cellular Probes* 7:293–299.

Madise, N. J., Matthews, Z. and Margetts, B. (1999). Heterogeneity of child nutritional status between households: A comparison of six sub-Saharan African countries. *Population Studies* 53(3): 331-43.

Maiden, M. C. J., Bygraves, J. A., Feil, E., Morelli, G., Russell, J. E., Urwin, R., Zhang, Q., Zhou, J., Zurth, K., Caugant, D. A., Feavers, I. M., Achtman, M. and Spratt, B. G. (1998). Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. In: *Proceedings of the National academy of Science of the United States of America* 95(6): 3140-3145.

Mangia, A. H., Duarte, R., Silva, L. A., Bravo, V. L. and Leal, M. C. (1993). Aetiology of acute diarrhoea in hospitalized children in Rio de Janeiro City, Brazil. *Journal of Tropical Pediatric* 39: 365-367.

Marquis, G. S., Ventua, G., Gilamn, R. H., Porras, E., Miranda, E., Carbajal, L. and Pentafiel, M. (1990). Faecal contamination of shanty town toddlers in households non-corralled poultry, Lima, Peru. *American Journal of Public Health* 80: 146-149.

Mattila, L., Siitonen, A., Kyronseppa, H., Simula, I., Oksanen, P. and Stenvic, M. (1992). Seasonal variation in etiology of travelers' diarrhoea Finnish-Moroccan study group. *Journal of Infectious Diseases* 165: 385-388.

Mazurier, S., van de Giessen, A., Heuvelman, K. and Wernars, K. (1992). RAPD analysis of Campylobacter isolates: DNA fingerprinting without the need to purify DNA. *Letters to Applied Microbiology*14: 260–262.

Mdegela, R. H., Nonga, H. E., Ngowi, H. A. and Kazwala, R. R. (2006). Prevalence of thermophilic campylobacter infection in humans, chickens and Crows in Morogoro, Tanzania. *Journal of Veterinary Medicine* B 53: 116-121.

Meinersmann, R. J., Helsel, L. O., Fields, P. I. and Hiett, K. L. (1997). Discrimination of *Campylobacter jejuni* isolates by *fla* gene sequencing. *Journal of Clinical Microbiology* 35: 2810–2814.

Meinersmann, R. J., Patton, C. M., Evins, G. M., Wachsmuth, K. I. and Fields, P. I. (2002). Genetic diversity and relationships of *Campylobacter* species and subspecies. *International Journal of Systematic and Evolutionary Microbiology* 52: 1789–1797.

Ministry of Health (1997). *Policy implications of adult morbidity and mortality: End of Phase 1 Report.* Ministry of Health, Dar es Salaam, Tanzania. 30pp.

Misawa, N., Allos, B. M. and Blaser, M. J. (1998). Differentiation of *Campylobacter jejuni* serotype O19 strains from non-O19 strains by PCR. *Journal of Clinical Microbiology* 36: 3567–3573.

Misawa, N., Kawashima, K., Kondo, F., Kushima, E., Kushima, K. and Vandamme, P. (2002). Isolation and characterization of Campylobacter, Helicobacter and

Anaerobiorillum strains from a puppy with bloody diarrhoea. *Veterinary Microbiology* 87: 353-364.

Missawa, N., Shinohara, S., Satoh, H., Itoh, H., Shinohara, K., Shimomura, K., Kondo, F. and Itoh, K. (2000). Isolation of Campylobacter species from zoo animals and polymerase chain reaction-based random ampliefied polymorphism DNA analysis. *Veterinary Microbiology* 71: 59-68.

Miwa, N., Takegahara, Y., Terai, K., Kato, H. and Takeuchi, T. (2003). *Campylobacter jejuni* contamination on broiler carcasses of *C. jejuni*-negative flocks during processing in a Japanese slaughterhouse. *International Journal of Food Microbiology* 84: 105–109.

Mondon, P., Thélu, J., Lebeau, B., Ambroise-Thomas, P. and Guillot, R. (1995). Virulence of *Aspergillus fumigatus* strains investigated by random amplified polymorphic DNA analysis. *Journal of Medical Microbiology* 42: 299–303.

Monteville, M. R. and Konkel, M. E. (2002). Fibronectin-facilitated invasion of T84 eukaryotic cells by *Campylobacter jejuni* occurs preferentially at the basolateral cell surface. *Infection and Immunity* 70: 6665–6671.

Mooney, A., Clyne, M., Curran, T., Doherty, D., Kilmartin, B. and Bourke, B. (2001). *Campylobacter upsaliensis* exerts a cytolethal distending toxin effect on HeLa cells and T-lymphocytes. *Microbiology* 147: 735–743.

Mooney, A., Byrne, C., Clyne, M., Johnson-Henry, K., Sherman, P. and Bourke, B. (2003). Invasion of human epithelial cells by *Campylobacter upsaliensis*. *Cell Microbiology* 5: 835–847.

Moser, I., Schroeder, W. and Salnikow, J. (1997). *Campylobacter jejuni* major outer membrane protein and a 59-kDa protein are involved in binding to fibronectin and INT 407 cell membranes. *FEMS Microbiology Letters* 157: 233–238.

Nachamkin, I. and Blaser M. J. (Eds) (2000). *Campylobacteriosis. 2nd edition.* American Society for Microbiology, Washington. 545pp.

Nachamkin, I., Allos, B. M. and Ho, T. (1998). *Campylobacter* species and Guillain-Barré syndrome. *Clinical Microbiology Review* 11: 555-67.

Nachamkin, I., Ung, H. and Patton, C. M. (1996). Analysis of the HL and O serotypes of *Campylobacter* strains by the flagellin gene typing system. *Journal of Clinical Microbiology* 34: 277–281.

Nadeau, E., Messier, S. and Quessy, S. (2002). Prevalence and comparison of genetic profiles of Campylobacter strains isolated from poultry and sporadic cases of campylobacteriosis in humans. *Journal of Food Protection* 65(1): 73-8.

Nacem, K. and Macaulay, M. E. (1983). Antibiotic resistance in *Campylobacter jejuni/coli* from human and animal sources. *Journal of Pakistan Medical Association* 33:91-94.

Nonga, H. E. (2005). Epidemiology of thermophilic campylobacters in humans, chickens and water in Morogoro, Tanzania. Dissertation for award of MVM degree at Sokoine University of Agriculture, Morogoro, Tanzania. 118p.

Noordhuizen, J. P. T. M., Thrusfield, M. V., Frankena, K. and Graat, E. A. M. (Eds.) (2001). Application of Quantitative Methods in Veterinary Epidemiology. Wageningen Press, Wageningen. 429pp.

Oberhelman, R. A and Taylor, D. N. (2000). Campylobacter infections in developing countries. In: *Campylobacteriosis, 2nd edition (Edited by Nachamkin. I. and Blaser M. J.),* American Society for Microbiology, Washington. pp 139-153.

On, S. L. W. (1996). Identification Methods for Campylobacter, Helicobacter and Related Organisms. *Clinical Microbiology Review* 9: 405-422.

Ooesterom, J., Nortamans, S., Karman, H. and Engels, G. M. (1983). Origin and prevalence of *Campylobacter jejuni* in poultry processing. *Journal of Food Protection* 46: 339-344.

Owen, R, J., Fayos, A., Hernandez, J. and Lastovica, A. (1993). PCR-based restriction fragment length polymorphism analysis of DNA sequence diversity of flagellin genes of *Campylobacter jejuni* and allied species. *Molecular and Cellular Probes* 7: 471–480.

Pearson, A. D., Greenwood, M., Healing, T. D., Rollins, D., Shamahat, M., Donaldson, J. and Colwell, R. R. (1993). Colonization of broiler chicken by waterborne *Campylobacter jejuni. Applied and Environmental Microbiology* 59(4): 987-996.

Pei, Z. and Blaser, M. J. (1993). PEB1, the major cell-binding factor of *Campylobacter jejuni*, is a homolog of the binding component in gram-negative nutrient transport systems. *Journal Biology and Chemistry* 268: 18717–18725

Penner, G. A., Bush, A., Wise, R., Kim, W., Domier, L., Kasha, K., Laroche, A., Scoles, G., Molnar, S. J. and Fedak, G. (1993). Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. *PCR Methods Application* 2: 341–345.

Penner, J. L. (1988). The genus *Campylobacter*: a decade of progress. *Clinical Microbiology Review* 1: 157-172

Perlman, D. M., Ampel, N. M., Schifman, R. B., Cohn, D. L., Patton, C. M., Aguirre, M. L., Wang, W. L. and Blaser, M. J. (1988). Persistence *Campylobacter jejuni* infection in patients infected with human immunodeficiency virus (HIV). *Annals of Internal Medicine* 108: 540-546.

Pickett, C. L. (2000). *Campylobacter* toxins and their role in pathogenesis In: *Campylobacter (Edited by Nachamkin, I., Blaser M. J),* ASM Press, Washington. pp. 179–190.

Piddock, L. J. (1995). Quinolones resistance and Campylobacter spp. *Journal of. Antimicrobial Chemotherapy* 36: 891-898.

Prescott, J. F. and Bruin-Mosch, C. W. (1981). Carriage of *Campylobacter jejuni* in health and diarrhoeic animals. *American Journal of Veterinary Research* 42: 164-165.

Purdy, D., Buswell, C. M., Hodgson, A. E., McAlpine, K., Henderson, I. and Leach, S. A. (2000). Characterization of cytolethal distending toxin (CDT) mutants of *Campylobacter jejuni. Journal of Medical Microbiology* 49: 473–479

Raji, M. A., Adekeye, J. O., Kwaga, J. K. and Bale, J. O. (2000). Bioserogroups of *Campylobacter* species isolated from sheep in Kduna state, Nigeria. *Small Ruminant Research* 37: 215-21.

Rani, R., Pramanik, A. K. and Das, U. (2004). Role of exposure to domestic animals in the etiology of campylobacter enteritis in children of rural community. *Indian Veterinary Journal* 81: 971-973.

Rao, M. R., Naficy, A. B., Savarino, S. J., Abu-Elyazeed, R., Wierzba, T. F. and Peruski, L. F, (2001). Pathogenicity and convalescent excretion of *Campylobacter* in rural Egyptian children. *American Journal of Epidemiology* 154: 166-73.

Rollins, D. M. and Colwell, R. R. (1986). Viable but nonculturable stage of *Campylobacter jejuni* and its role in survival in natural aquatic environments. *Applied and Environmental Microbiology* 52: 531-538.

Rosef, O. and Kapperud, G. (1983). Houseflies (*Musca domestica*) as possible vectors of *Campylobacter fetus* subsp. *jejuni*. *Applied and Environmental Microbiology* 45:381-383.

Rosef, O. (1981). The occurrence of *Campylobacter fetus* subspecies *jejuni* and *Salmonella* bacteria in some wild birds. *Nordic Veterinary Medicine Journal* 33: 539-543.

Rozynek, E., Dzierzanowska-Fangrat, K., Jozwiak, P., Popowski, J., Korsak, D. and Dzierzanowska, D. (2006). Poultry as a potential source of *Campylobacter jejuni* and *Campylobacter coli* infections in children in Poland. [http://www.blackwellpublishing.com/eccmid16/ abstract.asp?id=50848] site visited on 2/5/2008.

Saleha, A. A., Mead, G. C. and Ibrahim, A. L. (1998). *Campylobacter jejuni* in poultry production and processing in relation to public health. *World Poultry Science Journal* 54: 50-57.

Saleha, A. A. (2004). Epidemiological study on the colonization of chickens with Campylobacter in Broiler Farms in Malaysia: Possible Risk and Management Factors. *International Journal of Poultry Science* 3 (2): 129-134.

Sandberg, M. (2002). Epidemiological aspects to be considered for Salmonella and Campylobacter risk assessments in Norway. Thesis for award of Doctor Medicinae Veterianriae at Norwegian School of Veterinary Science, Oslo, Norway. 164pp.

Siemer, B. L., Harrington, C. S., Nielsen, E. M., Borck, B., Nielsen, N. L., Engberg, J. and On, S. L. W. (2004). Genetic relatedness among *Campylobacter jejuni* serotyped isolates of diverse origin as determined by numerical analysis of amplified fragment length polymorphism (AFLP) profiles. *Journal of Applied Microbiology* 96: 795–802.

Shane, S. M., Montrose, M. S. and Harrington, K. S. (1985). Transmission of Campylobacter jejuni by housefly (*Musca domestica*). *Avian Diseases* 29: 384-390.

Skelly, C. and Weinstein, P. (2003). Pathogen Survival Trajectories: An Eco-Environmental Approach to the Modeling Human Campylobacteriosis Ecology. *Environmental Health Perspectives* 111 (1): 19-26.

Skirrow, M. B. and Blaser, M. J. (1992). Clinical and epidemiological considerations. In *Campylobacter jejuni: Current status and future trends (edited by Nachamkin, I., Blaser, M. J., and Thompkins, L. S),* ASM Press, Washington D.C. pp. 3-8.

Skirrow, M. B. and Butzler, J.P. (2000). *Foreword. Campylobacter.* 2nd edition, ASM Press, Washngton DC. 239pp.

Skirrow, M. B. (1991). Epidemiology of Campylobacter enteritis. *International Journal of Food Microbiology* 12: 9-16.

Skirrow, M. B. (1987). A demographic survey of *Campylobacter. Salmonella* and *Shigella* infection in England. *Epidemiology and Infection* 99: 647-657

Skirrow, M. B. (1992). Campylobacter and Helicobacter. In: *Medical Microbiology 14th Edition (Edited by Greewood D., Slack, R. C. B. and Pentherer, J. F.),* Longman, London. pp. 353-358.

Skirrow, M. B. (1977). *Campylobacter* enteritis: a "new" disease. *British Medicine Journal 2*: 9-11.

Smith, J. L. (1996). Determinants that may be involved in virulence and disease in *Campylobacter jejuni. Journal of Food Safety* 16: 105-139.

Steinbrueckner, B., Haerter, G., Pelz, K. and Kist, M. (1999). Routine identification of *Campylobacter jejuni* and *Campylobacter coli* from human stool samples. *FEMS Microbiology Letters* 179: 227-232.

Stepniak, E., Zagalska M. and Switonski, M. (2002). Use of RAPD technique in evolution studies of four species in the family Canidae. *Journal of Applied Genetics* 43: 489-499.

Stern, N. J. (1992). Reservoir for *Campylobacter jejuni* and approaches for intervention in poultry. In: *Campylobacter jejuni: Current status and future trends (Edited by Nachamkin, I., Blaser M. J. and Tompkins L. S., Eds),* American Society of Microbiology, Washington. pp. 49-60.

Stern, N. J. (1994). Mucosal competitive exclusion to diminish colonization of chickens by *Campylobacter jejuni. Poultry Science* 73: 402-407.

Tauxe, R. V. (1992). Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In: *Campylobacter jejuni: Current status and future trends. (Edited by Nachamkin, I., Blaser M. J, and Thompkins L. S),* American Society for Microbiology, Washington. pp. 9-19.

Tauxe, R.V., Deming, M. S., Blake, P. A. (1985). *Campylobacter jejuni* infection on college campuses: A national survey. *American Journal of Public Health* 75: 659-660.

Taylor, D. N. (1992). Campylobacter infections in Developing countries. *In: Campylobacter jejuni: Current status and future trends. (Edited by Nachamkin, I., Blaser M. J., Tompkins L. S.),* American Society for Microbiology, Washington. pp. 20-30.

Taylor, D. N., Perlman, D. M., Echeverria, P. D., Lexomboon, U., Blaser, M. J. (1993). *Campylobacter* immunity and quantitative excretion rates in Thai children. Journal *of Infectious Diseases* 168: 754-758.

Taylor, D. N., Echeverria, P., Pitarangsi, C., Seriwatana, J., Bodhidatta, L. and Blaser, M. J. (1998). Influence of strain characteristics and immunity on the epidemiology of *Campylobacter* infections in Thailand. *Journal of Clinical Microbiology* 26: 863–868.

Thrusfield, M. (1995). *Veterinary Epidemiology. Second edition.* Blackwell Ltd., 483pp.

Trachoo, N. (2003). *Campylobacter jejuni*: An emerging pathogen. Sonklanakarin *Journal of Science and Technology* 25(1): 141-157.

United Republic of Tanzania (2002). 2002 Population and housing census [http://www.tanzania.go.tz/census/districts/morogorourban.htm] site visited on 13/6/2007.

Uzoigwe, C. (2005). Campylobacter infections of the pericardium and myocardium. *Clinical Microbiology and Infection* 11 (4): 253-5.

van Koningsveld, R., Rico, R., Gerstenbluth, I., Schimitz, P. I., Ang, C. W., Merkies, I. S., Jacobs, B. C., Alabi, Y., Endtz, H. P., van der Meche, F. G. and van Doorn, P. A. (2001). Gastroenteritis-associated Guillain-Barré Syndrome on the Caribbean island Curacao. *Neurology* 56: 1467-72.

van de Giessen, A., Mazurier, S. I., Jacobs-Reitsma, W., Jansen, W., Berkers, P., Ritmeester, P. and Werners, K. (1993). Study in Epidemiology and Control of Campylobacter jejuni in Poultry broiler flocks. *Applied and Environmental Microbiology* 58: 1913-1917.

Vandenberg, O., Dediste, A., Vlaes, L., Ebraert, A., Rotore, P. and Douat, N. (2001). Prevalence and clinical features of non-*Campylobacter jejuni/coli* species and related organisms in stool specimens (Abstract P-22). *International Journal of Medical Microbiology* 291 (31): 144.

Wassenaar, T. M. and M. J. (1999). Pathophysiology of *Campylobacter jejuni* infections of humans. *Microbes and Infection* 1: 1023–1033.

Wassenaar, T. M: and Newell, D. G. (2000). Genotyping of *Campylobacter* spp. *Applied and Environmental Microbiology* 66(1): 1–9.

Wassenaar, T. M., On, S. L. W. and Meinersmann, R. J. (2000). Genotyping and the consequences of genetic instability. *In: Campylobacter, 2nd Edition (Edited by Nachamkin, I., Blaser, M. J.)*, ASM Press, Washington, DC. pp. 369-380.

Welsh, J. and McClelland, M. (1990). Fingerprinting genome using PCR with arbitrary primers. *Nucleic Acid Research* 18: 7213-7218.

Wickin, H. V., Thwaites, R. T. and Frost, J. A. (2001). Drug resistance in Campylobacter species in England and Wales, 1993-2001 (AbstractB-05). *International Journal of Medical Microbiology* 291 (31): 6.

Williams, J. G. K., Hanafey, M. K., Rafalski, J. A. and Tingey, S. V. (1993). Genetic analysis using random amplified polymorphic DNA markers. *Methods in Enzymology* 218: 704–740.

Wooldridge, K. G. and Ketley, J. M. (1997). *Campylobacter*-host cell interactions. *Trends in Microbiology* 5: 96–102.

Workman, S. N., Mathison, G. E. and. Lavoie, M. C. (2005). Pet Dogs and Chicken Meat as Reservoirs of *Campylobacter* spp. in Barbados. *Journal of Clinical Microbiology* 43 (6): 2642–2650.

Yogasundram, K., Shane, S. M. and Harrington, K. S. (1989). Prevalence of *Campylobacter jejuni* in selected domestic and wild birds in Lousiana. *Avian Diseases* 33:664-667.

8.0 APPENDICES

Appendix 1: Human specimen submission form

HUMAN SAMPLE FORM

Sample No.. ..... Date: .....

Regional Hospital ☐ Mafiga ☐ Upendo ☐ SUA ☐ Other: .....

Patient's name: ..... Origin/Area of residence: .....

Age: ..... years ..... Months

Sex: Male ☐ Female ☐

Main symptom: Diarrhoeic ☐ Non-diarrhoeic ☐

Keeping of chickens? At home ☐ Neighbourhood ☐ Not at all ☐

Other animals kept at home/neighbourhood?

Cattle ☐ Sheep ☐ Goats ☐

Other poultry ☐ Pigs ☐ Dogs ☐ Cats ☐

Is drinking water boiled/treated? Yes ☐ No ☐

Laboratory diagnosis/ results:

Campylobacter culture: Positive ☐ Negative ☐

Appendix 2: Chicken sample collection form

CHICKEN SAMPLE FORM

Flock No. ..... Date: .....

Owner: ..... Place .....

Chicken population size: ....

Chicken types: Broilers ☐ Indigenous ☐ Layers ☐ Cockerels ☐

Chickens age: ..... weeks

Recent complaint?..... Last treated with ..... When treated? .....

Other animals kept at home/neighbourhood?

Cattle ☐ Sheep ☐ Goats ☐

Other poultry ☐ Pigs ☐ Dogs ☐ Cats ☐

Are there any children < 5 years old?

At home ☐ Neighbourhood ☐

Do the children access chicken houses? Yes ☐ No ☐

Is drinking water for human consumption boiled/treated? Yes ☐ No ☐

Laboratory diagnosis/ results:

Campylobacter culture: Positive ..... ☐ Negative ..... ☐